Neocortical Lewy Body Counts Correlate with Dementia in the Lewy Body Variant of Alzheimer's Disease

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Abstract. Patients with the Lewy body variant (LBV) of Alzheimer's disease (AD) meet diagnostic criteria for AD but have a lighter burden of plaque and tangle AD pathology despite comparable dementia. We quantified neocortical Lewy bodies (LB) in LBV patients (n = 14) using anti-ubiquitin polyclonal antibody, selecting for quantification those neocortical regions with the highest densities of LB. Neocortical neurofibrillary tangles (NFT) and neuritic plaques were evaluated with thioflavin-S. A group of classical AD patients (n = 12), matched for disease duration, was also studied. For most of these cases, entorhinal neurofibrillary pathology had previously been assessed by applying a modification of the Braak and Braak AD staging protocol. Although LBV and AD groups had similar mental test scores when last evaluated prior to death, lower neocortical NFT and plaque counts and lower modified Braak stages were observed in LBV. Neocortical NFT counts correlated with impaired neuropsychological test performance in AD but not in LBV. Plaque counts did not correlate with mental status in either group. Lewy body concentrations in four neocortical areas correlated significantly with dementia severity in LBV. The association of AD lesions in the neocortex with dementia in LBV was comparatively weaker than that observed for LB concentrations. These findings suggest that neocortical LB combined with entorhinal NFT or subcortical Parkinson's disease-type pathology may equalize the degree of dementia seen in LBV with that encountered in classical AD.

Key Words: Alzheimer's disease; Dementia; Lewy bodies; Neocortex; Neurofibrillary tangles; Senile plaques.

INTRODUCTION

Hansen et al (1) documented that subcortical and neocortical Lewy bodies (LB) are present in 75% of a "plaque-only" or "plaque-predominant" form of Alzheimer's disease (AD) in which neocortical neurofibrillary tangles (NFT) are either absent or sparse. Patients with this "Lewy body variant" of AD (LBV) show impairment of mental status comparable to that seen in classical AD (2–4). Though dementia is the presenting complaint of LBV patients, they typically display some parkinsonian features, including masklike facies and rigidity (3).

It is difficult to account for the equivalence of dementia in AD and LBV since, in addition to fewer NFT in LBV, neocortical plaque counts are not significantly high, and may even be lower in the temporal lobe (3, 5). Thus LBV has less AD pathology, though there is one report (6) that plaques are more numerous in LBV than AD. Neocortical plaques cannot easily be invoked as an explanation for LBV dementia, however, since plaque frequency does not correlate well with premortem dementia severity (7, 8, 9). Neurofibrillary tangle frequen-
pathology among LBV patients was compared with that seen among non-demented Parkinson’s disease (PD) patients rather than, as we would prefer, with that seen among classical AD patients. We decided, therefore, to try to replicate this important and pioneering study and to compare clinical-pathologic correlations seen among LBV patients with those found in classical AD.

MATERIALS AND METHODS

Subjects

Autopsy tissue was taken from 26 patients diagnosed antemortem as “probable AD” (or Lewy body variant of AD) according to NINCDS-ADRDA criteria (12) and later confirmed at postmortem to meet 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 (13, 14). Cases were obtained from the clinical series studied at the Alzheimer’s Disease Research Center (ADRC), University of California, San Diego. Those subsequently designated as LBV (n = 14) all had at least one LB in one or more subcortical nuclei—i.e., nBM, substantia nigra, or locus coeruleus—and at least one neocortical LB identified with hematoxylin and eosin (H&E)-stained preparations. We established these criteria (1, 2) in order to separate LBV both from pure AD, which lacks LB, and from pure Lewy body disease (i.e., diffuse Lewy body disease or idiopathic PD), which fails to meet NIA and CERAD criteria for a diagnosis of AD. In defining LBV, 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 underscore the actual pathology, since typical LBV cases have numerous neocortical and subcortical LB. Our definition of LBV requires the presence of subcortical LB on H&E-stained sections which obviates concern about overdiagnosis through misidentification of ubiquitin-positive gluteal neocortical NFT as LB. In the present study, one or more LB were found in the substantia nigra of 13 of the 14 LBV brains (93%). No nigral LB were found in any of the 12 AD specimens. The one LBV case lacking nigral LB had a LB in the substantia innominata and multiple neocortical LB, thus meeting our LBV inclusion criteria.

Selection of LBV cases from the larger pool available in the ADRC data set was based solely on availability of relatively complete neuropathological test results obtained within 30 months of death. The same criterion was applied to the selection of AD cases with the additional stipulation that these be comparable to the LBV in terms of estimated duration of disease (averaging 7 years). The age range for LBV patients was 66 to 86 years (mean = 78.7), while that for AD patients was 65 to 91 years (mean = 78.5). In order to assess the relative extent of neurofibrillar AD pathology manifested by patients in these two groups, we utilized data from a modification of the staging scheme of Braak and Braak (15) that had been previously applied to the LBV and AD cases and to six non-demented elderly controls. The six controls had undergone complete neuropsychological testing at the ADRC and ranged in age from 60 to 89 years (mean = 78.3).

Neuropsychological Tests

Results are reported for three commonly used assays of mental status: the memory-based Blessed Information-Memory-Concentration test (IMC, 16) as modified by Fuld (17) for American use, the cognitively more diverse Mini-Mental State Examination (MMSE) of Folstein (18), and the Dementia Rating Scale (DRS) of Mattis (19) which is comprised of subscales testing attention, conceptualization, construction, initiation, and memory. Errors are counted on the IMC, so its score varies directly with severity of dementia (range = 0 to 33). The other two scales count correct answers, so scores on the MMSE (range = 0 to 30) and DRS (range = 0 to 144) vary inversely with dementia severity. These psychometric tests were performed at a mean of 18 months before death in both AD and LBV cases. Selection of cases with relatively complete neuropsychological testing resulted in samples having a fairly broad range of mental status scores. On the MMSE, for example, the range was 0 to 20 among LBV patients and 4 to 19 among AD patients; the distribution of scores approximated normality in both groups. After the initial diagnosis of dementia, patients are often followed for 2 or more years at our ADRC prior to death and autopsy; their terminal MMSE scores may thus decline to zero, as was the case for two of the LBV patients.

Neuropathology

Some autopsies were performed within a few hours of death and others after overnight refrigeration, but postmortem intervals never exceeded 24 hours. At autopsy, the brains were divided sagittally 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 as previously described (20). Sections 7 μm thick were taken from these blocks and stained with H&E. Sections 10 μm thick were stained with thioflavin-S and viewed with ultraviolet light and 440 nm bandpass wavelength excitation filters to detect neuritic or diffuse plaques and NFT. Using other sections, we measured tissue reactivity to anti-synaptophysin (SY38, Boehringer-Mannheim, Indianapolis, IN), a monoclonal antibody against an integral protein component of the presynaptic terminal bouton, and visualized the degree of binding using the immunoperoxidase method. These observations could not be reliably quantitated, however, due to the fact that half of the tissue specimens were harvested from brains that had been in formalin for more than a year; the overfixed tissue had a lower and more variable anti-synaptophysin immunoreactivity than tissue embedded at the time of the original brain cutting.

Quantification of LB: Lewy bodies in brainstem nuclei were readily identified with H&E preparations. Neocortical LB, however, are more subtle, since they are usually less eosinophilic, less well defined, and generally lack a halo. Immunolabeling with antibody to ubiquitin clearly demonstrates these inclusions as recently described (21), and allows them to be accurately
quantified (22). Accordingly, paraffin sections 10 μm thick were immunolabeled with rabbit polyclonal affinity-purified anti-ubiquitin antibody (Chemicon, Temecula, CA). This antibody also labels NFT, but these fibrillary structures are generally distinguishable from labeled LB because the latter are round, nonfibrillary, 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 (globule NFT) and may be indistinguishable from LB (23). Discrimination of these lesions is to some extent facilitated by the fact that LB predominate in the deeper neocortical layers 5 and 6 whereas NFT prefer the more superficial layers 3 and 5.

A single observer (WS) quantified ubiquitin-immunolabeled LB in four areas previously found to be sites of predilection for LB in the neocortex (3): midfrontal (MF), cingulate (C), superior temporal (ST), and inferior parietal (IP). Tissue slides from neuropathologically pure AD were randomly intermixed with those from LBV cases in a blinded assessment so as to evaluate the contaminating influence of globule NFT. Single sections from each neocortical area were scanned initially with a 10× 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 25× 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 40× with the structure still centered. A systematic search was made of the tissue within the span of a 40× field (0.1 mm²) 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 the diameter of one 40× field. If the initial lower-power scans of the cortical ribbon did not disclose any LB-appearing targets, then the densest-staining region available was selected as the target and the above procedures applied. The count for a given 40× field was the number of LB contained within it. Counts were made in two separate regions per slide and then, after all slides had been examined, were repeated in three regions per slide. The first two counts were averaged and correlated with the average of the last three counts to provide an index of test-retest reliability, which was acceptably high within each neocortical area (n = 26; r range = 0.76 to 0.92, p < 0.001). Finally, the average of all five counts was computed as the LB score for each case in a particular neocortical area, and these were the LB data utilized in the analyses which follow. The code which concealed the identity of cases as LBV or AD was not broken until all LB counts were completed.

Figure 1 shows the frequency distribution of LB-like structures seen in LBV and AD groups. Since each case had an LB score for each of four neocortical regions, the 14 LBV cases would yield 56 such scores while the 12 AD cases would yield 48. At the time of LB quantification, however, some tissue blocks were missing from the inferior parietal area, so LB counts could not be made in this region for two LBV and three AD cases. The available LB scores therefore numbered 54 for LBV and 45 for AD cases. Among the former, the distribution peaked at 1.6 to 2.0 per field, with higher counts being relatively rare. Among AD cases the distribution of "LB" was severely skewed, with 62.2% of counts in the 0–0.4 range and 26.7% in the 0.4–0.8 category; by contrast, only 14.8% of the counts among LBV cases were in each of these categories. Combining counts above 2.0 as a single category, a chi-square comparison confirmed that the distributions of counts within the LBV and AD groups differed significantly (chi-square with 5 degrees of freedom = 40.0, p < 0.001). Even when the counts above 2.0 were deleted from the analysis, the difference between the distributions remained highly significant (chi-square with 4 degrees of freedom = 24.1, p < 0.001). These data imply that the lesions being counted among LBV cases were qualitatively different from those being counted among AD cases; specifically, we maintain that the lesions were comprised primarily of LB among the former and entirely of globule NFT among the latter. As a check on this reasoning, we selected the six AD patients who manifested the greatest number of LB-like inclusions in neocortical neurons. None of these six had any parkinsonian features on their last neurological examination, and they did not differ significantly from the remaining six AD patients in terms of MMSE, IMC, or DRS scores or NFT or plaques in the midfrontal, cingulate, superior temporal, or inferior parietal regions. Sections from these regions were immunostained with anti-ubiquitin antibody followed by thioflavin-S counterstaining, as previously described (24). For each case, a serial section from each sampled region was immunolabeled with anti-Alz 50 antibody (generously supplied by Dr Peter Davies) as a marker of NFT. For diligent searching, at least two neuronal inclusions resembling LB were identified on at least one anti-ubiquitin-stained slide from each case that could with certainty also be identified on a serial section immunostained with Alz 50. In the limited number of lesions meeting all these criteria, the LB-appearing inclusions were confirmed as thioflavin-S-positive under fluorescent microscopy.

Fig. 1. Number of observations made of counts (per 0.1 mm²) of LB-like structures seen in four neocortical regions. The total available number of LB counts was 45 for 12 patients with classical AD and 54 for 14 patients with LBV. Counts for LBV cases were primarily of LB, whereas those for AD cases were of globule NFT (see text).
and as Alz 50-positive on a serial section viewed under light microscopy. All observations were made using a 40× objective.

Quantification of Plaques and NFT: Quantifications of plaques and NFT were available from routine examination (by LH) of all brain tissue coming to our laboratory. On sections stained with thioflavin-S, plaques were counted using a 10X objective and NFT using a 40× objective on a Zeiss fluorescent microscope as previously described (1, 2). No tissue blocks were missing at the time of this quantification, so average NFT and total, diffuse, and neuritic plaque counts were available for all cases in all neocortical areas. Descriptively, diffuse plaques were irregularly shaped, amorphous or polymorphous zones of thioflavin positivity without neuritic disruption, lacking distinct boundaries or sharp edges. Immature neuritic plaques were generally spherical and brightly stained with well-defined borders; they contained filamentous amyloid and swollen neurites but no compact amyloid cores. Mature neuritic plaques were spherical and well circumscribed; they had dense, compact, round amyloid cores with peripheral coronas of dilated neurites. Only neuritic plaque counts (combining immature and mature forms) were utilized in the present study.

AD Neuropathology Staging: In order to make standardized comparisons of the extent of AD neurofibrillary pathology between AD, LBV, 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). Our modification of the Braak and Braak protocol is modeled closely on the original and attempts to parallel its six developmental stages of neurofibrillary pathology in AD: Stages I and II, the transentorhinal; Stage III and IV, the limbic; Stages V and VI, the neocortical. In our modification, NFT are quantified in layer two of the entorhinal cortex at the level of the mammillary bodies, where neuronal clusters give rise to the perforant path. Using thioflavin-S preparations, we count tangles in at least five neuron clusters in lamina two and average the results to give each brain a mean entorhinal cortex layer two neuron cluster tangle count. 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 tangle counts range from 0 to 3, and there are scattered tangles in the adjacent transentorhinal cortex. In brains assigned to Stage II, entorhinal layer two tangle 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 V, some neocortical regions may lack NFT, but such lesions must be present in moderate densities in at least two neocortical sections. In Stage VI, all neocortical sections must have at least three tangles per high power field in selected regions of the slide.

Statistical Analyses

Data were analyzed with a Statview (25) program package which calculated Pearson product-moment correlations, or r values, and compared mean differences between LBV and AD groups by t-test. All significant levels were two-tailed (p < 0.05).

RESULTS

Subject Characteristics

Demographic and clinical features of LBV and AD patients are summarized in Table 1. There were no significant differences between these groups in terms of age at death, proportion of males versus females, years of education or disease duration, or months elapsed between the last neuropsychological testing and death. In addition to making the foregoing comparisons, we checked for clinical features that would be anticipated to distinguish LBV from pure AD on the basis of previous findings (3). Case numbers were scrambled to permit blind scoring of eight parkinsonian features noted at the time of each patient’s last complete neurological examination prior to death. These features consisted of bradykinesia, masked facies, hypophonia, decreased speed and regularity of repetitive movements, rigidity and cogwheeling, postural tremor, stooped posture, and parkinsonian gait; all were rated as either present (=1) or absent (=0). No neuropsychological examination record was available for one LBV and one AD patient, both of whom had received their neuropsychological testing at another facility prior to the transfer of records to our ADRC. The number of parkinsonian features was greater for LBV patients (mean = 3.3) than for pure AD (mean = 0.5), and the difference was highly significant [r(22) = 3.81, p < 0.001]. Eight of the 11 pure AD cases for whom neurological examinations were available had no parkinsonian features.
Fig. 2. Number of LBV and pure AD patients and nondemented elderly controls classified at each of seven stages of AD NFT pathology, as modified from the six-stage system devised by Braak and Braak (15). The overall number of cases for which staging was available in each group was as follows: LBV (n = 12), AD (n = 6), controls (n = 6). Pure AD cases were all classified at Stages V and VI, whereas LBV predominated at Stages III and IV (p = 0.0008 by exact test). Controls were classified entirely at Stages 0, I, or II, significantly below the LBV cases (p = 0.0001).

whatsoever; one had three features and two had one each. All of the LBV patients had parkinsonian features.

Finally, we compared the modified Braak stages for AD neurofibrillary pathology which had previously been assigned to 12 of the LBV, six of the AD, and six nondemented elderly control brains. Figure 2 shows these data in bar graph format. Pure AD patients clustered at the most advanced modified Braak stages (6/6 at Stages V and VI) while LBV patients predominated at the more moderate levels (2/12 at Stages V and VI, 10/12 at Stages III and IV), and this difference was highly significant (p = 0.0008 by exact test). The LBV patients, in turn, had a more advanced modified Braak stage (0/12 at Stages 0, I, or II) compared to normal controls (6/6 at Stages 0, I, or II); this difference, too, was highly significant (p = 0.0001). Modified Braak scores were unavailable for two of the LBV and six of the AD cases because these brains had been processed prior to the publication of the original Braak staging protocol, and we were not yet taking the crucial section of entorhinal cortex. Because blocks were taken by several investigators for use in other projects, this entorhinal section was also missing in archival tissue from these eight cases.

Mean Scores on Neuropsychological Tests and on Physical Measures

Mean neuropsychological test scores in Table 2 indicate an equivalent degree of dementia in LBV and AD groups on each of the three mental status measures. For NFT and plaques, the two traditional measures of AD severity, LBV had significantly fewer NFT in all neocortical areas (p < 0.05) and also tended to have lower plaque counts, but the difference attained significance only in the IP area. “Lewy body” counts for the AD group are placed in parentheses because these lesions were almost certainly globose NFT; their mean frequency ranged from 0.18 to 0.55 per field and was in all neocortical regions significantly lower than that observed for true LB in the LBV group. A two-way analysis of variance was performed to compare LB counts among LBV with globose NFT counts among AD cases across the four neocortical regions. This analysis only included cases for which data were complete in all four regions, so n = 12 for LBV and n = 9 for AD. The overall mean LB count was 1.46 for LBV and 0.33 for AD, with the main effect for LBV vs AD being highly significant [F(1, 19) = 10.76, p < 0.004]. The main effect for neocortical subregion was negligible [F(3, 57) = 1.11] as was the interaction term [F = 1.33]. We therefore estimate that, at most, 22% of neocortical LB counted in LBV cases using only anti-ubiquitin staining are in fact globose NFT. The 22% figure is most likely an overestimate because, as Table 2 indicates, the AD have three to six times as many NFT per 40X field than do the LBV. A more realistic estimate would be that 5% of neocortical LB counted in LBV cases are globose NFT.

Correlations Between LB Counts and Mental Status

As shown in Table 3, among LBV cases all relationships between LB and mental status were in the anticipated direction and most were significant (p < 0.05). None of the correlations between mental status and the globose NFT seen in AD cases attained significance in the expected direction. As mentioned in the legend to Table 3, one of these correlations—between the DRS score and globose NFT in the IP cortex—was significant in a counterintuitive direction. That is, its direction suggests that a greater number of LB in the IP cortex is associated with better cognition. Such a relationship seems clearly spurious and most parsimoniously attributable to a statistical artifact created by the skewed distribution of pseudo-LB counts among AD cases (see Fig. 1).

Correlations Between AD Lesions and Mental Status

Table 4 shows that neocortical NFT counts were not significantly correlated with neuropsychological test scores among LBV cases, which is unsurprising since most LBV is “plaque-only” AD. Among AD cases NFT counts in several areas correlated significantly with mental status (p < 0.05). A few additional correlations between NFT counts and mental status among AD patients were of marginal significance in Table 4 (absolute r = 0.50 to 0.55, p < 0.10) while no correlations even ap-
TABLE 2
Mean Scores on Neuropsychological and Physical Measures

<table>
<thead>
<tr>
<th></th>
<th>LBV Mean ± SD</th>
<th>AD Mean ± SD</th>
<th>( p ) diff</th>
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<tbody>
<tr>
<td>Mental status</td>
<td></td>
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</tr>
<tr>
<td>MMSE (0–30 correct)</td>
<td>10.6 ± 7.2</td>
<td>10.4 ± 4.6</td>
<td>n.s.</td>
</tr>
<tr>
<td>IMC (0–33 errors)</td>
<td>20.9 ± 5.8</td>
<td>24.2 ± 5.2</td>
<td>n.s.</td>
</tr>
<tr>
<td>DRS (0–144 correct)</td>
<td>66.0 ± 22.0</td>
<td>66.1 ± 30.5</td>
<td>n.s.</td>
</tr>
<tr>
<td>NFT</td>
<td></td>
<td></td>
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<tr>
<td>Midfrontal</td>
<td>0.36 ± 0.8</td>
<td>1.92 ± 2.1</td>
<td>0.05</td>
</tr>
<tr>
<td>Cingulate</td>
<td>0.86 ± 0.9</td>
<td>2.75 ± 1.4</td>
<td>0.005</td>
</tr>
<tr>
<td>Superior temporal</td>
<td>1.21 ± 2.7</td>
<td>4.08 ± 2.7</td>
<td>0.02</td>
</tr>
<tr>
<td>Inferior parietal</td>
<td>0.86 ± 1.6</td>
<td>5.00 ± 5.4</td>
<td>0.05</td>
</tr>
<tr>
<td>Plaques</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Midfrontal</td>
<td>31.0 ± 13.4</td>
<td>40.1 ± 11.2</td>
<td>n.s.</td>
</tr>
<tr>
<td>Cingulate</td>
<td>19.7 ± 9.6</td>
<td>28.4 ± 13.3</td>
<td>n.s.</td>
</tr>
<tr>
<td>Superior temporal</td>
<td>24.0 ± 12.0</td>
<td>31.1 ± 9.7</td>
<td>n.s.</td>
</tr>
<tr>
<td>Inferior parietal</td>
<td>29.2 ± 12.1</td>
<td>38.1 ± 8.0</td>
<td>0.05</td>
</tr>
<tr>
<td>LB</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Midfrontal</td>
<td>1.71 ± 1.3</td>
<td>(0.20) ± (0.26)</td>
<td>(0.0007)</td>
</tr>
<tr>
<td>Cingulate</td>
<td>1.86 ± 1.2</td>
<td>(0.27) ± (0.25)</td>
<td>(0.0001)</td>
</tr>
<tr>
<td>Superior temporal</td>
<td>1.41 ± 1.1</td>
<td>(0.55) ± (0.48)</td>
<td>(0.02)</td>
</tr>
<tr>
<td>Inferior parietal</td>
<td>1.22 ± 1.2</td>
<td>(0.18) ± (0.19)</td>
<td>(0.02)</td>
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For LBV \( n = 14 \) except for inferior parietal LB, where \( n = 12 \). For AD \( n = 12 \) except for inferior parietal "LB", where \( n = 9 \). For "LB" counts on pure AD cases the mean and standard deviation have been placed in parentheses to signify that these are globule NFT. The "\( p \) diff" column shows the significance of \( t \) tests comparing LBV and AD. Mental status measures are the Mini-Mental State Examination (MMSE), the Blessed Information Memory Concentration test (IMC), and the Dementia Rating Scale (DRS); they are described in the text. Plaques were counted under a 10× objective while NFT and LB were counted under a 40× objective.

Proached significance among LBV patients. Neuritic plaque counts were not significantly correlated with neuropsychological test scores among either LBV or AD patients, so these data are not shown in Table 4.

**DISCUSSION**

Replicating the results of one prior study (11) we found that neocortical LB concentrations in LBV were associated with dementia severity. Our findings confirm and extend those previously reported in that (a) all patients received the same set of well-validated mental status measures premortem, (b) clinical-pathological correlations were not exaggerated by the inclusion of data from normal controls or an overrepresentation of severely impaired patients, (c) correlations were of the parametric Pearson type rather than the less sensitive Spearman rank order type, and (d) the results for LBV patients were contrasted with those for patients with classical AD rather than for patients with PD. Our results are consistent with research described earlier (2–4) in showing that, compared to AD cases closely matched on biographical variables and dementia severity, LBV patients have fewer classical AD lesions and a lower Braak stage (see Tables 1 and 2 and Fig. 2). Also consistent with prior work is our finding that plaque frequency is unrelated to degree of dementia in either group (7–9). Neocortical NFT frequency correlated with mental status in AD but not in LBV (see Table 4) while LB frequency correlated with dementia in LBV (see Table 3). It is possible that NFT in the entorhinal cortex and hippocampus, which develop earlier than neocortical NFT in AD, can account for some of the dementia in LBV, since medial temporal lobe NFT density correlates with dementia in the pathologically similar condition of PD combined with AD (26). LBV and a pathologically similar condition designated senile dementia of the Lewy body type (27) have entorhinal and hippocampal neurofibrillary pathology intermediate in severity between pure AD and age-matched controls (28). The modified Braak stage comparisons shown in Figure 2 are entirely consistent with these prior findings and make the further point that while all LBV cases met CERAD plaque-based criteria for AD, they are also clearly distinguishable from non-demented controls in terms of entorhinal neurofibrillary pathology.

Our findings relating the frequency of neocortical LB to dementia in LBV are complicated only slightly by the discovery that, in a blinded quantification of LB using anti-ubiquitin staining of neocortical tissue, some LB-like structures are seen in neuropathologically pure AD. As has been thoroughly documented elsewhere (23),
TABLE 3
Correlations Between LB Counts and Mental Status in LBV Cases

<table>
<thead>
<tr>
<th>Mental status measures</th>
<th>MMSE</th>
<th>IMC</th>
<th>DRS</th>
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<tr>
<td>Midfrontal (MF)</td>
<td>-0.43</td>
<td>0.48</td>
<td>-0.48</td>
</tr>
<tr>
<td>Cingulate (C)</td>
<td>-0.54*</td>
<td>0.62**</td>
<td>-0.64**</td>
</tr>
<tr>
<td>Superior temporal (ST)</td>
<td>-0.62**</td>
<td>0.76***</td>
<td>-0.46</td>
</tr>
<tr>
<td>MF + C + ST</td>
<td>-0.63**</td>
<td>0.74***</td>
<td>-0.63**</td>
</tr>
<tr>
<td>Inferior parietal (IP)</td>
<td>-0.42</td>
<td>0.53</td>
<td>-0.54</td>
</tr>
<tr>
<td>MF + C + ST + IP (n = 12)</td>
<td>-0.56</td>
<td>0.66***</td>
<td>-0.63*</td>
</tr>
</tbody>
</table>

*p < 0.05.
**p < 0.025.
***p < 0.01.
N = 14.
Mental status measures are described in Table 2 and in the text. For pure AD cases, the *r* coefficients for correlations between globose NFT counts and mental status measures ranged from -0.57 to 0.36 in MF, C, ST; and MF + C + ST (where n = 12) and from -0.59 to 0.72 in IP and MF + C + ST + IP (where n = 9). The only correlation that achieved statistical significance in AD cases was between the DRS score and globose NFT in IP (*r* = 0.72, *p* < 0.05). This correlation is in a counterintuitive direction and seems most parsimoniously attributable to a statistical artifact created by the highly skewed distribution of globose NFT counts among AD cases (see text and Fig. 1).

anti-ubiquitin staining is valuable for visualizing neocortical LB but will occasionally bind to other structures in a manner that gives a false appearance of LB in a very small number of neurons. The distribution of globose NFT in AD was strongly skewed toward zero and was manifestly different from the distribution of true LB in LBV. A limited sampling of globose NFT on sections having the greatest number of these rare structures in AD cases confirmed that they had NFT-like properties, staining positively with anti-Alz 50 antibody and fluorescing after staining with thioflavin-S. We estimate that about 5% of the LB counted among LBV patients were false-negative globose NFT; a fairly constant error rate of this sort would not have influenced the magnitude of the correlations found in our analyses of the relationship between LB and dementia.

Despite significant correlations between LB counts and neuropsychological test scores, some authorities contend that the absolute numbers of LB are simply too small to ascribe to them a causal role in dementia. Our LB counting techniques are non-random and select for quantification those regions of neocortex with the highest lesion densities, which could exaggerate the apparent frequency of LB. However, exactly the same techniques have been applied to the quantification of NFT in this and other studies. The mean number of anti-ubiquitin-labeled LB per 0.1 mm² among the LBV is of the same order of magnitude as the mean number of thioflavin-S-labeled NFT among pure AD cases, particularly in the midfrontal cortex. Midfrontal NFT correlate significantly with neuropsychological test performance and are accepted as an important contributor to AD dementia (8, 9, 29). Logically, LB frequency should be given the same consideration as a potential factor in LBV dementia. Indeed, the correlations reported here could actually underestimate the relationship between LB and dementia if, during the inevitable delay between final neuropsychological testing and death, LB continue to accumulate while mental status declines even further. We did not, however, find the interval (in days) between final testing and death to be appreciably correlated with LB frequency in any neocortical region.

We also examined the possibility that some unmeasured third factor might underlie both LB formation and cognitive impairment. Table 1 compares LBV and AD patients on several demographic variables that could be candidate third factors; of these, only the number of parkinsonian clinical features differed significantly between

TABLE 4
Correlations Between NFT Counts and Mental Status

<table>
<thead>
<tr>
<th>Mental status measures</th>
<th>MMSE</th>
<th>IMC</th>
<th>DRS</th>
</tr>
</thead>
<tbody>
<tr>
<td>LBV cases only</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Midfrontal (MF)</td>
<td>-0.26</td>
<td>0.24</td>
<td>-0.01</td>
</tr>
<tr>
<td>Cingulate (C)</td>
<td>0.18</td>
<td>-0.23</td>
<td>0.44</td>
</tr>
<tr>
<td>Superior temporal (ST)</td>
<td>-0.26</td>
<td>0.27</td>
<td>0.01</td>
</tr>
<tr>
<td>Inferior parietal (IP)</td>
<td>-0.26</td>
<td>0.16</td>
<td>-0.16</td>
</tr>
<tr>
<td>MF + C + ST + IP</td>
<td>-0.22</td>
<td>0.18</td>
<td>0.04</td>
</tr>
<tr>
<td>AD cases only</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Midfrontal (MF)</td>
<td>-0.47</td>
<td>0.36</td>
<td>-0.59*</td>
</tr>
<tr>
<td>Cingulate (C)</td>
<td>-0.51</td>
<td>0.47</td>
<td>-0.58*</td>
</tr>
<tr>
<td>Superior temporal (ST)</td>
<td>-0.21</td>
<td>0.26</td>
<td>-0.22</td>
</tr>
<tr>
<td>Inferior parietal (IP)</td>
<td>-0.61*</td>
<td>0.50</td>
<td>-0.55</td>
</tr>
<tr>
<td>MF + C + ST + IP</td>
<td>-0.62*</td>
<td>0.54</td>
<td>-0.63*</td>
</tr>
</tbody>
</table>

For LBV n = 14. For AD n = 12. Mental status measures are described in Table 2 and in the text.
*p < 0.05.
the two groups. In addition to this clinical overlap between PD and LBV, there may even be shared genetic features (30). Parkinson’s disease is always associated with LB formation, and sometimes with dementia, but our LBV patients did not have clinical PD. They presented to the ADRC with dementia, not parkinsonism, and many did not develop their parkinsonian features until months or years after the initial examination. Some neocortical LB are seen in most patients with idiopathic PD and may, when sufficiently numerous, be a factor in the dementia seen in a quarter of PD patients (23, 28, 31). These LB-containing neurons stain for tyrosine hydroxylase (32, 33). Neocortical dopamine depletions and dementia could result from the loss of these neurons, though neocortical neurotransmitter deficits associated with dementia might alternatively be attributable to subcortical neuron loss and LB in the nBM, locus coeruleus, or substantia nigra.

Another candidate third factor that could contribute to dementia in LBV is neocortical synapse loss (5), since such loss correlates with impaired mental status in AD (7, 29, 34). Masliah et al (5) found equivalent neocortical and hippocampal synapse density loss in LBV and AD relative to controls, which could explain the comparable dementia in LBV and AD. Zhou et al (35) found that neocortical synapse loss, as indexed by anti-synaptophysin immunoreactivity, was greater in demented than in non-demented PD patients, while Nishimura et al (36) reported anti-synaptophysin immunolabeling of neocortical and subcortical LB. Conceivably, the LB is a marker for disturbed cytoskeletal function which results in impaired synaptic precursor transport, synapse loss, and consequent dementia. As noted earlier, we were unable to quantify neocortical synapse density as part of this study. Our laboratory has previously reported (37) a correlation between dementia severity and synapse density in the hippocampal formation among LBV patients but a lesser degree of absolute synapse loss among LBV relative to AD.

The results of this study imply that neocortical LB, or neurodegenerative processes associated with their formation, contribute to dementia in LBV. Other investigators have shown that AD neurofibrillary pathology in the medial temporal lobe, as measured by Braak staging, also correlates with cognitive impairment (26). Finally, neocortical neuritic plaques may cause synaptic loss, and decreased synapse density is yet another dementia correlate (7). Some of these neuropathologic abnormalities occur in PD and pure diffuse Lewy body disease, others in conventional AD, and all of them in LBV. What, then, is the relationship between LBV and AD, on the one hand, and LBV and PD, on the other? Our concept of LBV is that it represents a neurodegenerative hybrid separate and distinct from AD and PD but with clinical, neuropathological, and neurochemical features of both. To conceptual-ize LBV, we draw upon an analogy. Just as a mule is neither horse nor donkey, despite being composed of nothing other than horse and donkey, so too is LBV a “different animal” than AD or PD, despite sharing features with both. This mulish analogy may seem fanciful or strained, but the hybridization parallels the genetic level. Like AD, but unlike PD, LBV has an increased apolipoprotein-E e-4 allele frequency (38). As in PD, but not in AD, the cytochrome p450 CYP2D6 debrisoquine-4 hydroxylase mutant B allele gene is increased in LBV (30). Clinical, neuropathologic, neurochemical, and genetic data can therefore all be interpreted as supporting a neurodegenerative hybridization paradigm. The plethora of competing appellations applied to brains which we consider LBV, however, testifies eloquently to the absence of nosologic unanimity in the neuropathology of dementias associated with Lewy bodies.

REFERENCES


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