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Perception of Fragmented Letters by Patients With Pathologically Confirmed Dementia With Lewy Bodies or Alzheimer Disease

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Abstract

Background and Objective
Patients with dementia with Lewy bodies perform worse than those with Alzheimer disease (AD) on tests of visual perception, but the clinical utility of these tests remains unknown because studies often had clinically diagnosed groups that may inadvertently cross-contaminate Lewy body disease (LBD) with pure AD pathology, used experimental tests not easily adaptable for clinical use, and had no way to examine relationships between the severity of LBD pathology and degree of cognitive impairment. Therefore, we sought to determine whether performance on a widely used clinical test of visuoperceptual ability effectively differentiates between patients with autopsy-confirmed LBD or AD and correlates with the severity of LBD pathology.

Methods
Patients with mild to moderate dementia (n = 42) and cognitively healthy controls (n = 22) performed a Fragmented Letters Test in which they identified letters of the alphabet that were randomly visually degraded by 70% and additional visuospatial and episodic memory tests. At autopsy, dementia cases were confirmed to have LBD (n = 19), all with concomitant AD, or only AD (n = 23). Severity of α-synuclein pathology in the hippocampus and neocortex was rated on an ordinal scale.

Results
Patients with LBD performed worse than those with AD (B = −2.80 ± 0.91, $p = 0.009$) and healthy controls (B = −3.34 ± 1.09, $p = 0.01$) on the Fragmented Letters Test after adjustment for age, sex, education, Mini-Mental State Examination score, and ability to name intact letters. Patients with AD did not differ from controls (B = −0.55 ± 1.08, $p = 0.87$). The test effectively distinguished between patients with LBD or AD with 73% sensitivity and 87% specificity, and the area under the curve in receiver operating characteristic analyses was 0.85 (95% CI 0.72–0.95), higher than for standard tests of visuospatial ability (Block Design; 0.72; CI 0.35–0.75) or memory (California Verbal Learning Test, trials 1–5; 0.55; CI 0.57–0.88). Fragmented Letters Test scores were negatively correlated with LBD pathology density ratings in hippocampus and neocortical regions (Spearman $r_s = -0.53$ to -0.69).

Discussion
Fragmented Letters Test performance can effectively differentiate patients with LBD pathology from those with only AD pathology at a mild to moderate stage of dementia, even when LBD occurs with significant concomitant AD pathology, and may also be useful for gauging the severity of cortical α-synuclein pathology in those with LBD.

Glossary

AD = Alzheimer disease; **ADNC** = AD neuropathologic change; **ADRC** = AD Research Center; **CVLT** = California Verbal Learning Test; **DLB** = dementia with Lewy bodies; **DRS** = Dementia Rating Scale; **FBS** = fetal bovine serum; **H&E** = hematoxylin & eosin; **LBD** = Lewy body disease; **MCI** = mild cognitive impairment; **MMSE** = Mini-Mental State Examination; **NC** = normal controls; **PCA** = posterior cortical atrophy; **PDD** = Parkinson disease dementia; **POD** = Pfeffer Outpatient Disability; **ROC** = receiver operating characteristic; **TRIS** = tris(hydroxymethyl)aminomethane; **UCSD** = University of California, San Diego; **UPDRS** = Unified Parkinson's Disease Rating Scale.

Lewy body disease (LBD) refers to a class of neurodegenerative disorders pathologically characterized by cell loss and deposition of abnormal intracytoplasmic aggregates of α-synuclein (i.e., Lewy bodies and Lewy neurites) in brainstem nuclei, limbic regions, and neocortex.[1-3] Two expressions of LBD are associated with the development of dementia: dementia with Lewy bodies (DLB) and Parkinson disease dementia (PDD). Both DLB and PDD are characterized by prominent cognitive impairment (e.g., mild cognitive impairment [MCI] or dementia), motor symptoms of Parkinson disease (e.g., bradykinesia, gait disturbances, and tremor), and behavioral symptoms (e.g., hallucinations, REM sleep disorder, and fluctuations in attention) and differ primarily in the order of symptom onset with dementia developing concurrently or before motor symptoms in DLB and at least 1 year after the onset of motor symptoms in PDD. The co-occurrence of Alzheimer disease (AD) pathology with LBD is extremely common, particularly when LBD manifests as DLB.[4-6] This overlap is acknowledged in the proposed clinicopathologic criteria for DLB[2] wherein the likelihood of the typical DLB clinical presentation increases with increasing LBD pathology but decreases with increasing concomitant AD pathology (holding LBD pathology constant). The criteria suggest that "if abundant neocortical neuritic plaques and tangles are present in addition to Lewy bodies, the clinical profile may more closely resemble AD rather than DLB" (pg. 89).[2]

Similarity in the dementia syndromes associated with LBD and AD has led to a search for features that might help to distinguish between the 2 pathologies during life, a particularly important endeavor given the nascent state of α-synuclein biofluid and imaging biomarker development. A promising direction of search is the disproportionately severe deficit in visuospatial abilities in DLB and PDD compared with AD.[6-8] Patients with DLB or PDD have been shown to perform worse than those with AD on tests of visual object perception,[9-12] visual search,[13,14] visual motion discrimination,[15] visual texture recognition,[16] copying or drawing 2-dimensional figures,[17-19] and constructing 3-dimensional objects (i.e., Block Design).[20-22] These unexpectedly severe visuospatial deficits are apparent despite similar levels of global dementia across patient groups. The clinical utility of these measures remains unknown, however, because these studies often had clinically defined groups that may inadvertently cross-contaminate LBD and pure AD pathology, used tests that were protracted experimental measures not easily adaptable for clinical use, and had no way to determine the severity of LBD pathology and its relationship to the degree of cognitive impairment the tests revealed. Therefore, the goal of this study was to determine whether a rapid and easily administered test of visuoperceptual ability, the Fragmented Letters Test from the Visual Object and Space Perception Battery,[23] effectively differentiates between patients with autopsy-confirmed LBD or AD and whether performance on this test is associated with the severity of LBD pathology.

Methods

Participants

Patients with MCI or dementia who were eventually confirmed at autopsy to have LBD (n = 19) or AD (n = 23) were included in this study. All patients had been participants in the University of California, San Diego (UCSD) Shiley-Marcos AD Research Center (ADRC) through which they received yearly physical, neurologic, and neuropsychological assessments. Eligible participants met the following inclusion criteria: (1) autopsy revealed no significant pathologic process (e.g., hippocampal sclerosis, metabolic encephalopathy, or infarct with a clinical history of stroke) other than LBD or AD; and (2) a comprehensive behavioral, motor, and neuropsychological battery, including the Fragmented Letters Test, had been completed at one of the annual evaluations. A group of cognitively healthy elderly individuals (n = 22) who served as normal controls (NCs) in the UCSD ADRC and completed the Fragmented Letters Test at one of the annual evaluations was included for comparison with the patient groups.

The mean age, years of education, and scores on the Mini-Mental State Examination (MMSE) and the Mattis Dementia Rating Scale (DRS) for the 3 groups at the time of the Fragmented Letters Test evaluation are shown in Table 1. The 19 patients with pathologically confirmed LBD had received a clinical diagnosis of probable DLB (n = 4, 21%), PDD (n = 3, 16%), probable AD (n = 9, 47%), or MCI (n = 3, 16%) based on the ADRC evaluation at which the Fragmented Letters Test had been administered (diagnosing neurologists were blind to Fragmented Letters Test results). The 23 patients with pathologically confirmed AD had all presented with an amnestic multidomain cognitive deficit profile and received a clinical diagnosis of probable AD (n = 17, 74%), probable DLB (n = 1, 4%), or amnestic multidomain MCI (n = 5, 22%).

Table 1 Participant Demographics and Cognitive Test Scores

	NC	AD (AD+ LBD−)	LBD (AD+ LBD+)	
	n = 22	n = 23	n = 19	p Value[a]
Demographics and global mental status tests				
Age (y)	75.2 (9.4)	80.3 (5.9)	72.6 (7.3)	<0.001
Education (y)	16.0 (2.8)	15.2 (3.0)	15.0 (2.5)	0.084
Sex (M/F; %M)	9/22 (41%)	13/23 (57%)	15/19 (79%)	0.052
APOE genotype (% ε4+)	27%	52%	63%	0.33
Test-death interval (y)	—	4.1 (1.8)	3.8 (2.0)	0.68
MMSE (30 points)	29.5 (0.7)	22.4 (3.8)	23.8 (4.4)	0.28
Mattis DRS (144 points)	140.9 (2.2)	115.0 (12.6)	112.8 (19.1)	0.66
POD (20 points)	0 (0)	10.1 (5.8)	9.2 (6.5)	0.61
UPDRS (motor; 108 points)	0.7 (2.8)	1.6 (3.7)	15.0 (15.3)	0.002
Visuoperceptual, visual construction, and memory tests				
Fragmented Letters (20 points)	19.5 (0.6)	19.0 (2.1)	14.9 (4.7)	0.002
Intact Letters (26 points)	25.9 (0.4)	25.9 (0.3)	25.4 (1.1)	0.052
Block Design Test (48 points)	46.4 (10.1)	27.3 (15.2)	15.2 (12.1)	0.008
CVLT trials 1–5 (80 points)	49.0 (13.5)	20.6 (8.9)	19.3 (11.6)	0.72

Abbreviations: AD = Alzheimer disease; CVLT = California Verbal Learning Test; DRS = Dementia Rating Scale; LBD = Lewy body disease; MMSE = Mini-Mental State Examination; POD = Pfeffer Outpatient Disability; UPDRS = Uniform Parkinson's Disease Rating Scale.
The mean (and SD) age, years of education, years between testing and death, and MMSE, Mattis DRS, and POD scores for the normal control (NC) participants and patients with autopsy-confirmed AD or LBD. The sex and APOE ε4 genotype distributions are also shown.
[a] p Value for LBD vs AD comparison.

Neuropathologic Examination and Diagnosis

The brain was divided sagittally, and then, the left hemibrain was fixed in 10% buffered formalin, and the right hemibrain was sectioned coronally and frozen at −70°C. The formalin-fixed left hemibrain was serially sectioned in 1 cm slices, and tissue blocks from midfrontal, inferior parietal, and superior temporal cortices, primary visual cortex, hippocampus, amygdala, basal ganglia, substantia nigra, and cerebellum were processed for histopathologic examination by hematoxylin and eosin (H&E), thioflavin S, and immunohistochemistry with antibodies to detect tau (PHF-1; Sigma-Aldrich), β-amyloid (ab69d, from Dr. Edward Koo), and α-synuclein (81A; BioLegend) deposits.

Brains were staged for the degree of neurofibrillary tangle pathology using the Braak staging scheme and neuritic plaque density using methods recommended by the Consortium to Establish a Registry for AD.[24] AD was operationalized using the NIA-Reagan consensus criteria for the postmortem diagnosis of AD, wherein Braak stage V–VI with moderately to severely dense neuritic plaques corresponds to a high likelihood that dementia is due to AD.[25] Lewy body pathology, identified by H&E staining and immunostaining with antibodies against α-synuclein, was classified as none (but possibly olfactory only), amygdala predominant, brainstem predominant, limbic, or neocortical subtypes proposed in consensus guidelines for the pathologic diagnosis of DLB[1,2,26] (Table 2). Hippocampal TAR DNA binding protein 43 (TDP-43) pathology had been examined by immunohistochemical staining (Proteintech#10782-2-AP polyclonal, 1:12,000) in only 13 AD (11 negative and 2 positive) and 2 LBD (1 negative and 1 positive) cases and was not further analyzed.

The 19 patients with pathologically confirmed LBD included 17 neocortical and 2 limbic. All the LBD cases had concomitant AD pathology, in most cases sufficient to warrant a secondary diagnosis of AD (historically called "Lewy body variant of AD"[7]). Concomitant AD neuropathologic change (ADNC) in patients with LBD was high in 10 (53%), medium in 3 (16%), and low in 6 (32%) (all could be considered AD+ LBD+). Cases were not classified as LBD if Lewy bodies were found only in the amygdala.

The 23 patients with pathologically confirmed AD included 18 with no Lewy bodies and 5 with sparse Lewy bodies only in the amygdala. None of the AD cases had Lewy bodies or abnormal α-synuclein immunostaining in the neocortex or pigmented brainstem nuclei (all could be considered AD+ LBD−). The degree of ADNC in the patients with AD was high in 20 (87%), medium in 2 (9%), and low in 1 (4%).

	AD (AD+ LBD−) n = 23	LBD (AD+ LBD+) n = 19
Age at death	84.4 (5.7)	76.5 (7.8)
Neurofibrillary tangle staging (Braak)		
Braak 0	0 (0%)	0 (0%)
Braak I–II	0 (0%)	5 (26%)
Braak III–IV	2 (9%)	4 (21%)
Braak V–VI	21 (91%)	10 (53%)
Neuritic plaque score (CERAD)		
No neuritic plaques	0 (0%)	3 (16%)
Sparse neuritic plaques	1 (4%)	1 (5%)
Moderate neuritic plaques	9 (39%)	11 (58%)
Frequent neuritic plaques	13 (57%)	4 (21%)
Aβ plaque score (Thal)		
Thal 0	0 (0%)	0 (0%)
Thal 1–2	0 (0%)	1 (5%)
Thal 3	0 (0%)	0 (0%)
Thal 4–5	16 (70%)	10 (53%)
Missing Thal	7 (30%)	8 (42%)
Lewy body staging (McKeith)		
Lewy bodies: none (or olfactory only)	18 (78%)	0 (0%)
Lewy bodies: amygdala predominant	5 (22%)	0 (0%)
Lewy bodies: brainstem predominant	0 (0%)	0 (0%)
Lewy bodies: limbic	0 (0%)	5 (26%)
Lewy bodies: neocortical	0 (0%)	14 (74%)

Abbreviations: AD = Alzheimer disease; CERAD = Consortium to Establish a Registry for AD; LBD = Lewy body disease.
Neuropathologic characteristics of patients with autopsy-confirmed AD or LBD. The number (and percentage) of participants in each group falling within various stages of neurofibrillary tangle, neuritic plaque, and Lewy body severity and distribution is shown.

Ratings of Lewy Body Density

Seven-micrometer sections were obtained from paraffin-embedded formalin-fixed tissue from the hippocampus and midfrontal, superior temporal, and inferior parietal neocortex. On day 1, the 7-μm sections were deparaffinized in xylene and rehydrated using graded ethanols, and then, antigen retrieval was performed using 88% formic acid for 5 minutes.[27] Slides were then placed in 30% H_2O_2 in methanol solution for 30 minutes, washed in 0.1 M tris(hydroxymethyl)aminomethane (TRIS) at 7.6 pH, blocked with 2% fetal bovine serum (FBS) in 0.1 M TRIS, and incubated overnight in primary antibody directed against phosphorylated α-synuclein (81A; BioLegend, 1:10,000) at 4°C. On the second day, after washing with 1% TRIS solution and blocking with 2% FBS, slides were incubated in biotinylated horse anti-mouse IgG or goat anti-rabbit IgG secondary antibody 1:1,000 (Vector Laboratories, Burlingame, CA) for 1 hour at room temperature and then for an additional 1 hour in avidin/biotin-based peroxidase (Vector Laboratories). The chromogen used was 3,3′-diaminobenzidine (Vector Laboratories) with hematoxylin as the counter stain. Slides were then dehydrated in graded ethanols, treated with xylene, and cover-slipped. The severity of α-synuclein pathology in each region was rated by a single investigator (D.G.C.) using a standard ordinal rating scale that included 0 (none), 1 (mild), 2 (moderate), and 3 (severe).[28] The rater was blind to cognitive performance of patients.

Clinical Evaluation and Diagnosis

The annual standardized clinical, neurologic, and neuropsychological evaluations have been previously described.[6] The clinical evaluation included a review of history with the patient and/or informant, mental status testing using the MMSE and DRS, Clinical Dementia Rating, assessment of psychiatric symptoms (e.g., depression and psychosis including hallucinations), and assessment of functional impairment using the Pfeffer Outpatient Disability (POD) Scale or the Functional Assessment Questionnaire (converted to corresponding POD scores). A structured neurologic examination was completed that included the motor portion of the Unified Parkinson's Disease Rating Scale (UPDRS) to assess parkinsonian features. Neuropsychological evaluation included administration of multiple standardized tests of memory, language, executive functions, attention, and visuospatial abilities.[6]

A consensus clinical diagnosis was made by 2 or more board-certified neurologists with expertise in dementia and movement disorders after reviewing the results of the annual evaluation. Diagnosing neurologists were informed whether the neuropsychological assessment identified deficits in 2 or more domains of cognition, but not of individual test or cognitive domain scores. Probable AD was diagnosed according to National Institute of Neurological and Communicative Disorders and Stroke - Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA)[29] or National Institute on Aging-Alzheimer's Association (NIA-AA) criteria.[30] Probable DLB was diagnosed according to published criteria based on the presence of dementia (that preceded mild parkinsonism) and at least 2 of 3 additional core features of mild parkinsonism, well-formed visual hallucinations, and fluctuations in consciousness or attention.[1,2] REM sleep behavior disorder was considered but had not been systematically assessed before its inclusion in the latest DLB guidelines.[2] PDD was diagnosed on the basis of the presence of at least 2 of the cardinal motor signs of Parkinson disease and objective cognitive deficits on neuropsychological tests and functional decline due to cognitive problems.[3] Motor signs had to precede cognitive decline by more than 1 year.

Figure 1 Fragmented Letters Test Scores
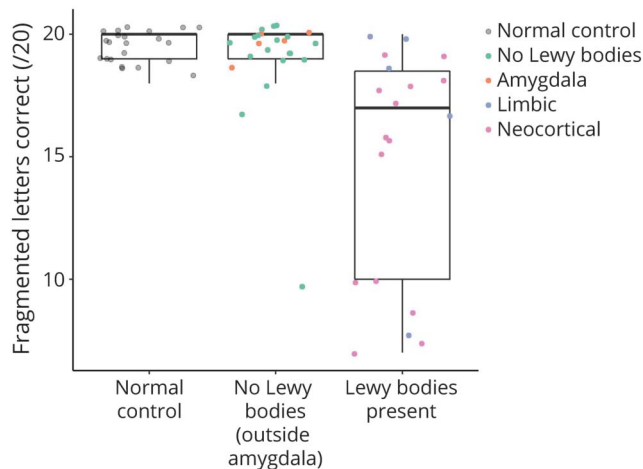
The distribution of scores achieved on the Fragmented Letters Test by normal control participants, patients with Alzheimer disease (AD) pathology and no Lewy body pathology (with [orange] or without [green] amygdala-only Lewy bodies), or patients with Lewy body pathology (limbic in blue and neocortical in pink) with or without concomitant AD pathology.

Visuoperceptual, Visual Construction, and Memory Tests

The Fragmented Letters Test and additional tests of visual construction (Block Design) and verbal memory (California Verbal Learning Test) were administered as part of the ADRC neuropsychological evaluation. Diagnosing neurologists and neuropathologists were blind to scores on these tests. Participants were tested individually in a quiet, well-lit room.

The Fragmented Letters Test

This test was adapted from the Incomplete Letters Test of the Visual Object and Space Perception Battery,[23] which is available from Pearson Assessments at pearsonclinical.co.uk/store/ukassessments/en/Store/Professional-Assessments/Cognition-%26-Neuro/Visual-Object-and-Space-Perception-Battery/p/P100009236.html. Participants were asked to identify 20 letters of the alphabet that were randomly visually degraded (i.e., fragmented) by 70%. Each fragmented letter was presented individually on an 18-inch computer monitor and remained visible until the participant stated a letter name. The response was recorded by the test administrator. The fragmented letters were presented in a fixed random order for all participants. Each fragmented letter was approximately 4 inches by 4 inches in size and shown in black on a white background in the center of the computer screen. The participant sat comfortably approximately 18 inches from the screen. Immediately after all fragmented letters had been shown, participants were asked to identify all 26 letters of the alphabet presented one at a time in a random order in their intact form in the same manner and size as the fragmented letters. The number of

Figure 2 Diagnostic Sensitivity and Specificity of Cognitive Test Scores
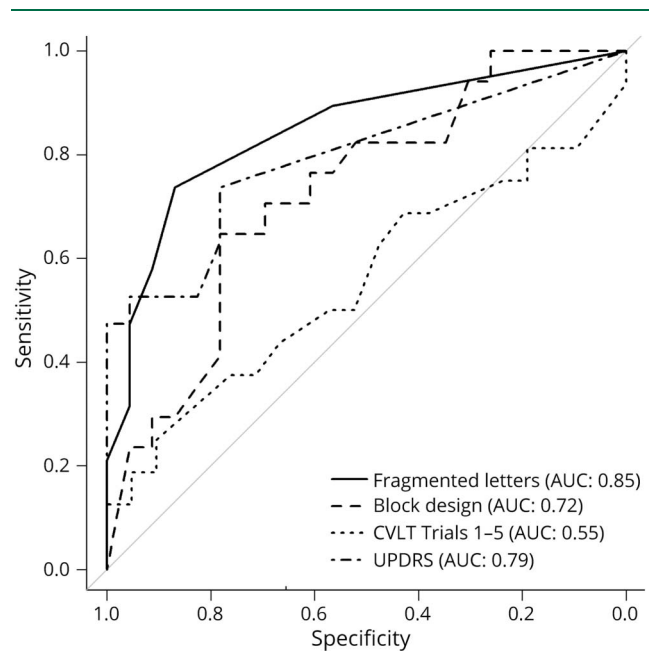
Receiver operating characteristic (ROC) curves comparing sensitivity and specificity for distinguishing between patients with dementia associated with autopsy-confirmed Lewy body disease or Alzheimer disease using scores from the Fragmented Letters Test, the Block Design Test, the California Verbal Learning Test (CVLT) measure of learning across 5 presentation-recall trials, and the motor portion of the Unified Parkinson's Disease Rating Scale (UPDRS). The area under the curve (AUC) for each test measure is also shown.

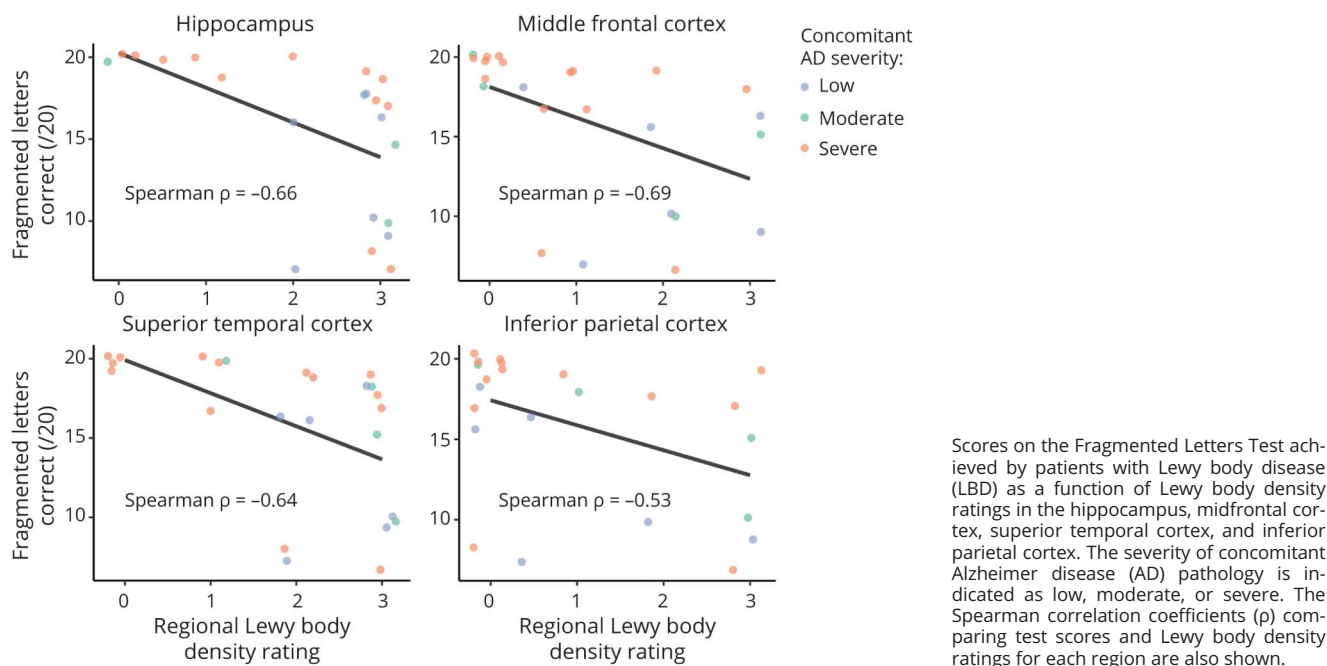correctly identified fragmented letters was the variable of interest in this study.

Block Design Test (Wechsler Intelligence Test for Children–Revised)

This is a test of visuospatial abilities that requires participants to construct a series of visual designs using 4 to 9 identical 1-inch by 1-inch blocks that have 2 red sides, 2 white sides, and 2 half-red, half-white sides. Each design must be constructed as quickly as possible. Possible scores on the test range from 0 to 62, with higher scores indicative of better performance. The total score on this test is a sensitive measure of visuospatial impairment in DLB.[21,31] The children's version was used because its level of difficulty is more appropriate for patients with dementia.

California Verbal Learning Test

This is a standardized word-list memory test that assesses the rate of learning, retention after short- and long-delay intervals, semantic encoding ability, and recognition memory. It consists first of 5 presentation/free recall trials for 16 items from 4 semantic categories to assess learning. Thereafter, short-(immediately after an interference list) and long-delay (20 minutes) free and cued recall are elicited, followed by a yes-no recognition test. The total number of words recalled across the 5 learning trials is a sensitive measure of learning and

Figure 3 Relationship Between Fragmented Letters Test Scores and Lewy Body Density

Scores on the Fragmented Letters Test achieved by patients with Lewy body disease (LBD) as a function of Lewy body density ratings in the hippocampus, midfrontal cortex, superior temporal cortex, and inferior parietal cortex. The severity of concomitant Alzheimer disease (AD) pathology is indicated as low, moderate, or severe. The Spearman correlation coefficients (ρ) comparing test scores and Lewy body density ratings for each region are also shown.

memory impairment in AD[32] or DLB[33,34] and is used as a variable of interest in this study.

Standard Protocol Approvals, Registrations, and Patient Consent

The research protocol was reviewed and approved by the human subjects review board at the University of California, San Diego. Informed consent to participate in the present investigation was obtained at the point of entry into the longitudinal study from all patients or their caregivers consistent with California State Law. Informed consent for autopsy was obtained at the time of death from the next of kin.

Statistical Analyses

Demographic and clinical characteristics were compared across groups using a 3-group analysis of variance for continuous variables with post hoc Tukey Honestly Significant Difference tests for significant results or a 3-group Fisher exact test for categorical variables with post hoc pairwise Fisher exact comparisons for significant results. Comparisons of Fragmented Letters Test scores across groups were performed using linear least squares regression adjusting for age, sex, education, level of dementia (i.e., MMSE score), and ability to name intact letters. We chose to control for these 5 covariates in our final model because they were potentially associated with performance on the Fragmented Letters Test, and this allowed a more precise isolation of the effect of group on test performance. Receiver operating characteristic (ROC) curve analyses were used to examine the ability of the Fragmented Letters Test to differentiate between patients with LBD or AD. The ROC curve analyses were also

completed with more complex visuospatial (i.e., Block Design) and memory (i.e., California Verbal Learning Test [CVLT]) tasks, and areas under the curve (AUCs) were compared. Associations between Fragmented Letters Test performance and severity of LBD pathology in various brain regions were assessed with Spearman rank-order correlation coefficients.

Data Availability

Anonymized data not published within this article will be made available by request from any qualified investigator.

Results

The LBD, AD, and NC groups differed significantly in age (F $[2,60]$ = 5.28; p = 0.008, $_p\eta^2$ = 0.15). Post hoc pairwise comparisons showed that patients with AD were older than patients with DLB (p = 0.0007), whereas neither patients with LBD (p = 0.24) nor those with AD (p = 0.25) differed in age from NC participants. The 3 groups did not differ in the level of education (F $[2,60]$ = 2.28; p = 0.11, $_p\eta^2$ = 0.07). The ratio of men to women did not differ across the groups (Fisher exact test p = 0.07), but there was a trend toward a higher ratio in the LBD group than in the other groups. The groups did not differ in proportions of APOE ε4+ individuals (Fisher exact test p = 0.08), but there was a trend for a higher proportion of APOE ε4+ individuals in the AD group than other groups. The groups differed on MMSE (F $[2,60]$ = 27.7; $p < 2.99 \times 10^{-9}$, $_p\eta^2$ = 0.48) and DRS (F $[2,60]$ = 29.8; $p < 1.02 \times 10^{-9}$, $_p\eta^2$ = 0.50) scores. Post hoc pairwise

Figure 4 Relationship Between Fragmented Letters Test Scores and Tau Pathology
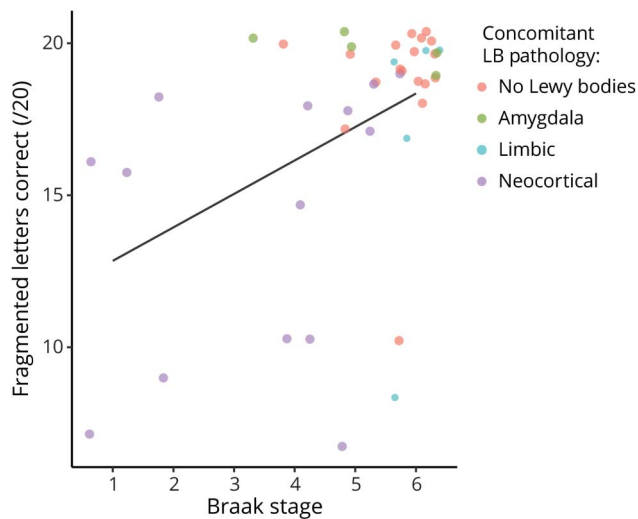
Scores on the Fragmented Letters Test achieved by patients with Lewy body disease (LBD) or Alzheimer disease (AD) as a function of Braak staging for tau pathology. Concomitant Lewy body pathology is indicated as no Lewy bodies, amygdala only, limbic, or neocortical.

comparisons showed that patients with LBD or AD performed worse than NC participants on both tests (p's < 0.05) but did not differ from each other (p = 0.38 for the MMSE; p = 0.84 for the DRS). The LBD and AD groups did not differ in the length of time between the Fragmented Letters Test evaluation and death (t [41] = 0.42; p = 0.68, d = 0.13) (Table 1).

Patients with LBD performed worse than those with AD (B = −2.80 ± 0.91, p = 0.009) and controls (B = −3.34 ± 1.09, p = 0.01) on the Fragmented Letters Test after adjustment for demographics (age, sex, and education), MMSE score, and ability to name intact letters (Figure 1 and Table 1). Patients with AD did not differ from controls on the Fragmented Letters Test (B = −0.55 ± 1.08, p = 0.87), although 1 patient with AD performed particularly poorly despite a typical amnestic multidomain cognitive deficit profile, only mild visuospatial impairment, mild dementia (MMSE = 27), and acceptable visual acuity (OD 20/70, OS 20/25).

The Fragmented Letters Test was able to distinguish between patients with LBD or AD with 73% sensitivity and 87% specificity at a maximally effective cutoff threshold of 18.5 points. ROC curve analyses showed that the Fragmented Letters Test was excellent in discriminating between patients with LBD or AD with an AUC of 0.85 (95% CI 0.72–0.95) (Figure 2). This AUC value was higher than for another commonly used test of visuospatial ability, the Block Design Test (AUC = 0.72; CI 0.56–0.88; 65% sensitivity and 78% specificity at a maximally effective cutoff threshold of 18.5 points), a sensitive episodic memory measure (learning across trials 1–5) from the CVLT (AUC = 0.55; CI 0.35–0.75; 25%

sensitivity and 90% specificity at a maximally effective cutoff threshold of 11 points), or the motor portion of the UPDRS (AUC = 0.79; CI 0.66–0.91).

Scores on the Fragmented Letters Test achieved by patients with LBD were negatively correlated with Lewy body pathology density ratings in the hippocampus (r_s = −0.66, p < 0.01), midfrontal cortex (r_s = −0.69, p < 0.001), superior temporal cortex (r_s = −0.64, p < 0.001), and inferior parietal cortex (r_s = −0.53, p = 0.01) (Figure 3). These associations were stronger than those between Lewy body pathology density ratings and Block Design (hippocampus: r_s = −0.56, p < 0.02, midfrontal cortex: r_s = −0.42, p > 0.05, superior temporal cortex: r_s = −0.57, p < 0.02, and inferior parietal cortex: r_s = −0.15, p > 0.50) or CVLT learning across trials 1–5 (hippocampus: r_s = −0.59, p < 0.01, midfrontal cortex: r_s = −0.25, p > 0.02, superior temporal cortex: r_s = −0.42, p > 0.05, and inferior parietal cortex: r_s = −0.17, p = 0.50) scores, particularly for density ratings in the inferior parietal cortex.

In contrast to its negative correlation with Lewy body pathology density ratings, Fragmented Letters Test performance was positively associated with the Braak stage across the entire sample (LBD and AD) (Figure 4, panel A) (r_s = 0.45, p = 0.003) and in the subsample with LBD and concomitant AD (Figure 4, panel B) (r_s = 0.51, p = 0.03). Thus, those patients with LBD with more AD pathology actually performed better on the Fragmented Letters Test than those with less concomitant AD pathology.

Discussion

Our results show that patients with mild to moderate dementia and autopsy-confirmed LBD and concomitant AD pathology perform worse than those with only AD pathology on a simple and easily administered visuoperceptual task, the Fragmented Letters Test. Patients with LBD were impaired on the task relative to cognitively healthy older adults, whereas patients with AD who were equally demented were unimpaired. Because the severity of AD pathology was greater in the AD group than in the LBD group, our results suggests that the LBD patients' deficit on the Fragmented Letters Test is primarily a reflection of α-synuclein pathology not present in those with only AD. This suggestion is supported by the significant negative correlations we observed between Fragmented Letters Test scores and the severity of Lewy body pathology in the hippocampus and neocortex of patients with LBD and by the negative correlation between the Braak stage (e.g., tangle pathology) and Fragmented Letters Test scores in those with Lewy body pathology. These results are consistent with a previous study that found worse Fragmented Letters Test performance in patients with clinically diagnosed DLB than in those with AD dementia[9] and extends these results to neuropathologically confirmed patient groups where the relationship between test performance and neocortical Lewy body pathology becomes evident.

Our results also suggest that the Fragmented Letters Test has excellent clinical utility for differentiating LBD from AD in mild to moderate stages of dementia; however, these results should be considered preliminary and need to be verified in an independent sample. Despite having similar levels of global dementia measured by mental status examinations or functional rating scales of activities of daily living, patients with LBD and concomitant AD pathology could be distinguished from those with only AD pathology, with 73% sensitivity and 87% specificity using a maximally effective cutoff score on the Fragmented Letters Test. This discriminative ability was higher than for a sensitive measure of episodic memory (i.e., the CVLT) and a standard test of visuospatial function (i.e., Block Design) in the same patients and was higher than that reported for other tests of visuoperceptual function in patients with clinically diagnosed DLB.[12,35,36] The ability to identify illusory contours (e.g., a white square outlined by black discs), for example, was significantly impaired in patients with DLB compared with those with AD dementia and distinguished between the conditions with 88.6% sensitivity but only 37.1% specificity.[35] The Newcastle visuoperception battery identified visuoperceptual deficits in 71% of patients with clinically diagnosed DLB using computer-based measures of angle discrimination, color discrimination, form discrimination, and motion perception but also identified these deficits in 40% of equally demented patients with AD dementia.[12] ROC curve analysis of scores on a visual texture discrimination task produced an area under the curve of 0.69 for distinguishing between patients with clinically diagnosed DLB or AD dementia[35] compared with an area under the curve of 0.85 for the Fragmented Letters Test in this study.

The ability to effectively distinguish between LBD and AD at mild to moderate stages of dementia is important for a number of reasons. From a prognostic perspective, there is evidence that global cognitive decline,[37] and specifically decline in executive functions and visuospatial abilities,[6] is faster in patients with LBD and concomitant AD pathology than in those with only AD pathology. In addition, the degree of visuospatial impairment is able to predict the rate of subsequent global cognitive decline[31] and the development of visual hallucinations[21] in those with LBD (with a DLB phenotype), but not in those with only AD. Thus, accurate clinical diagnosis is needed to provide reliable prognostic information to patients. From a treatment perspective, new drugs that target underlying AD pathology, such as Aβ-directed antibodies (e.g., aducanumab and donanemab) or tau-directed immunotherapy, may be less effective in individuals with LBD and concomitant AD pathology than in those with only AD pathology because the drugs only engage 1 aspect of the pathology that contributes to cognitive impairment. The ability to identify individuals less likely to benefit from a particular treatment has important implications for the design of clinical trials and choice of pharmacotherapy.

The physiologic basis of disproportionately severe visuoperceptual deficits in patients with LBD remains unknown but may be related to occipital cortex dysfunction that does not typically occur in patients with AD. The occipital cortex of patients with LBD can have white matter spongiform change with coexisting gliosis that is not present in those with AD,[38] and in some cases, there may be deposition of Lewy neurites[39] or Lewy bodies.[40] Hypometabolism[41] and decreased blood flow[42] occurs in the primary visual and visual association cortex on PET or SPECT neuroimaging in patients with DLB but not in those with AD. Patients with DLB had decreased theta band activity and higher alpha and beta band power during a visual, but not an auditory, event–related potential oddball task, suggesting that decreased theta and a lack of inhibition in alpha band power might be an oscillatory underpinning of high-level visual disturbances in LBD.[43] In addition, patients with DLB show decreased functional connectivity (relative to healthy controls) in visuoperceptual regions (middle and inferior occipital gyri, middle and inferior temporal gyri, and fusiform gyrus) activated on fMRI during the performance of a version of the Fragmented Letters Test.[44] The left middle occipital gyrus, medial occipital regions, and left fusiform gyrus have been shown to activate in healthy individuals when attending to single letters vs symbols, pseudoletters, or digits.[45,46]

It should be noted that a rare posterior cortical atrophy (PCA) subtype of AD exists that could be difficult to distinguish from LBD on the basis of the Fragmented Letters Test. A limitation of this study is that no atypical AD-related PCA cases were included. Patients with the PCA variant of AD have disproportionate atrophy and AD pathology in the occipital cortex and posterior parietal cortex relative to other cortical association areas[47] and exhibit a dementia syndrome dominated by visual dysfunction with prominent visual agnosia, constructional apraxia, visual field defects, decreased visual attention, impaired color perception, decreased contrast sensitivity, and features of Balint syndrome such as optic ataxia, gaze apraxia, and simultanagnosia.[48,49] PCA can generally be distinguished from LBD by its severe impairment of all visual functions, whereas LBD appears to predominantly affect higher-order visuospatial function while sparing lower complexity processes such as visual acuity or simple spatial orientation judgments.[50] This suggests that intact letter identification, which was not impaired in patients with LBD or AD in this study, might be impaired in patients with PCA and be useful for distinguishing between LBD and PCA, but this awaits further study. The most obvious differential is that memory and executive function abilities prominently affected in LBD are relatively preserved in the PCA variant of AD.[48,49]

Another limitation of this study is the relatively small sample size and the inability to match the DLB and AD cohorts on age. This is not unusual for an autopsy study that must foresee the potential value of a particular cognitive task and await neuropathologic diagnoses; however, the results clearly need to be replicated in a larger sample with better age matching. We attempted to account for the age difference, as much as possible, by including age as a factor in all models and analyses. Furthermore, all patients with AD had a multidomain

amnestic cognitive presentation, although those with atypical clinical presentations (e.g., PCA) were not explicitly excluded from the study. A potential limitation is that a few patients with PDD were included in the LBD group. Although there are differences in the cognitive manifestations of PDD and DLB that make including them in a single LBD group arguable,[6] both have prominent visuospatial dysfunction that might be detectable through the identification of fragmented letters. A larger study that compares patients with PDD and DLB manifestations of LBD on the Fragmented Letters Test would be useful. It is also a limitation that TDP-43 pathology was examined in very few individuals, so any effect of concomitant limbic-predominant age-related TDP-43 encephalopathy pathology on the identification of fragmented letters could not be determined. Finally, the density of LBD pathology was not examined directly in the occipital or occipital-temporal (e.g., fusiform gyrus) cortex that might contribute to letter identification but rather in regions where LBD pathology is most prominent and presumably most reflective of overall disease severity.

In summary, the Fragmented Letters Test is a rapid and easily administered visuoperceptual task that can effectively differentiate patients with LBD pathology from those with only AD pathology at a mild to moderate stage of dementia, even when LBD occurs with significant concomitant AD pathology and presents clinically as DLB or AD dementia. Furthermore, the performance of the Fragmented Letters Test may be useful for gauging the severity of cortical α-synuclein pathology in those with LBD.

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Disclosure

Publication History

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David P. Salmon, PhD	Department of Neurosciences, University of California	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; and analysis or interpretation of data

Appendix *(continued)*

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