




Cognitive and Pathological Influences of Tau Pathology in Lewy Body Disorders

David Coughlin, MD ^{1,2,3,6} Sharon X. Xie, PhD,^{8,9} Mendy Liang, BS,^{1,2} Andrew Williams, BS,^{1,2} Claire Peterson, BS,^{1,2} Daniel Weintraub, MD,^{1,6,10} Corey T. McMillan, PhD,^{1,3} David A. Wolk, MD,^{1,8} Rizwan S. Akhtar, MD PhD ^{1,6} Howard I. Hurtig, MD,^{1,6} H. Branch Coslett, MD,^{1,7} Roy H. Hamilton, MD MS,^{1,7} Andrew D. Siderowf, MD MSCE,^{1,6} John E. Duda, MD,^{1,10} Katya Rascovsky, PhD,^{1,3} Edward B. Lee, MD PhD,^{4,5,8} Virginia M.-Y. Lee, PhD,^{4,5,8} Murray Grossman, MD,^{1,3} John Q. Trojanowski, MD PhD,^{4,5,8} and David J. Irwin, MD MSTR ^{1,2,3}

Objective: To use digital histology in a large autopsy cohort of Lewy body disorder (LBD) patients with dementia to test the hypotheses that co-occurring Alzheimer disease (AD) pathology impacts the anatomic distribution of α -synuclein (SYN) pathology and that co-occurring neocortical tau pathology in LBDs associates with worse cognitive performance and occurs in a pattern differing from AD.

Methods: Fifty-five autopsy-confirmed LBD (Parkinson disease with dementia, $n = 36$; dementia with Lewy bodies, $n = 19$) patients and 25 AD patients were studied. LBD patients were categorized as having moderate/severe AD copathology (SYN + AD = 20) or little/no AD copathology (SYN–AD = 35). Digital measures of tau, β -amyloid (A β), and SYN histopathology in neocortical and subcortical/limbic regions were compared between groups and related to antemortem cognitive testing.

Results: SYN burden was higher in SYN + AD than SYN–AD in each neocortical region ($F_{1, 54} = 5.6\text{--}6.0$, $p < 0.02$) but was equivalent in entorhinal cortex and putamen ($F_{1, 43\text{--}49} = 0.7\text{--}1.7$, $p > 0.2$). SYN + AD performed worse than SYN–AD on a temporal lobe-mediated naming task ($t_{27} = 2.1$, $p = 0.04$). Antemortem cognitive test scores inversely correlated with tau burden ($r = -0.39$ to -0.68 , $p < 0.05$). AD had higher tau than SYN + AD in all regions ($F_{1, 43} = 12.8\text{--}97.2$, $p < 0.001$); however, SYN + AD had a greater proportion of tau in the temporal neocortex than AD ($t_{41} = 2.0$, $p < 0.05$), whereas AD had a greater proportion of tau in the frontal neocortex than SYN + AD ($t_{41} = 3.3$, $p < 0.002$). SYN + AD had similar severity and distribution of neocortical A β compared to AD ($F_{1, 40\text{--}43} = 1.6\text{--}2.0$, $p > 0.1$).

Interpretation: LBD patients with AD copathology harbor greater neocortical SYN pathology. Regional tau pathology relates to cognitive performance in LBD dementia, and its distribution may diverge from pure AD. Tau copathology contributes uniquely to the heterogeneity of cognitive impairment in LBD.

ANN NEUROL 2019;85:259–271

Lewy body disorders (LBDs), which include Parkinson disease (PD), PD with dementia (PDD), and dementia with Lewy bodies (DLB), are a clinically and pathologically

heterogeneous group of neurodegenerative diseases characterized by intracellular α -synuclein (SYN) Lewy pathology at autopsy.¹ Although the current clinical distinction

View this article online at wileyonlinelibrary.com. DOI: 10.1002/ana.25392

Received Aug 29, 2018, and in revised form Dec 4, 2018. Accepted for publication Dec 5, 2018.

Address correspondence to Dr Irwin, 3400 Spruce St, Department of Neurology, 3W Gates Building, Philadelphia, PA 19104.

Email: dirwin@penmedicine.upenn.edu

From the ¹Department of Neurology, Perelman School of Medicine at the University of Pennsylvania; ²Digital Neuropathology Laboratory, Perelman School of Medicine at the University of Pennsylvania; ³Frontotemporal Dementia Center, Perelman School of Medicine at the University of Pennsylvania; ⁴Department of Pathology and Laboratory Medicine, Perelman School of Medicine at the University of Pennsylvania; ⁵Center for Neurodegenerative Disease Research, Perelman School of Medicine at the University of Pennsylvania; ⁶Parkinson's Disease and Movement Disorders Center, Perelman School of Medicine at the University of Pennsylvania; ⁷Center for Cognitive Neuroscience, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; ⁸Alzheimer's Disease Center, Perelman School of Medicine at the University of Pennsylvania; ⁹Department of Biostatistics, Epidemiology and Informatics Perelman School of Medicine at the University of Pennsylvania; and ¹⁰Michael J. Crescenz VA Medical Center, Parkinson's Disease Research, Education, and Clinical Center, Philadelphia, PA, USA 19104

between PDD and DLB is based on the timing of dementia onset in relation to parkinsonism, this distinction is currently under debate^{2,3} due in part to the poor prediction of these clinical diagnoses for distinct pathologic substrates.^{4,5} The two disorders often share common motor signs, cognitive features, prodromal features such as rapid eye movement sleep behavior disorder, and genetic risk factors.⁶ Despite this overlap, there is also well-described heterogeneity in specific domains of cognitive impairment and presence or severity of distinct motor features across these conditions.^{7,8}

Detailed postmortem studies can provide insight into the underlying biological substrates of this variability as well as help define biologically meaningful patient subgroups that improve upon the current clinical distinction between PDD and DLB. Previous clinicopathological studies have found that regional distribution of SYN pathology may influence certain clinical features of LBDs, including the presence of hallucinations, the occurrence of dementia, and survival.^{9–12} However, the co-occurrence of clinically significant Alzheimer disease (AD)-associated tau and β -amyloid ($A\beta$) pathology is common and found in up to 50% of all LBDs.⁴ Although we and others have demonstrated that a higher burden of AD copathology is associated with decreased overall survival and faster progression to dementia even when adjusting for age,^{4,13,14} little is known regarding the relationship between specific, discrete cognitive features and the regional distribution of SYN and AD copathology in LBDs. This gap is due in part to the qualitative nature of traditional neuropathological staging systems that use limited ordinal estimates of pathological burden.

Here, we examine a large LBD dementia autopsy cohort with antemortem neuropsychological testing using digital histology to objectively measure pathologic burden in neocortical brain regions associated with cognition. Using this unique approach, we tested the hypothesis that tau and $A\beta$ copathology is associated with a greater burden of neocortical SYN pathology in LBDs. We previously found that tau pathology has a strong influence on the timing of onset of dementia in LBDs⁴; therefore, we hypothesized that tau is also an important contributor to the pattern of cognitive impairment in LBDs with dementia. Based on *in vitro* model data that suggests tau pathology may be cross-seeded by strains of pathological SYN¹⁵ detected in human LBD brains,¹⁶ we tested the hypothesis that tau in LBDs has a different neocortical distribution compared to “pure AD” pathology (ie, without neocortical SYN pathology).

Patients and Methods

Participants

Patients and data were abstracted from the University of Pennsylvania Integrated Neurodegenerative Disease

Database.¹⁷ Patients selected were clinically evaluated and followed at the University of Pennsylvania’s Parkinson’s Disease and Movement Disorder Clinic, Frontotemporal Dementia Center, Alzheimer’s Disease Core Center, or Michael J. Crescenz VA Medical Center’s Parkinson’s Disease Research, Education, and Clinical Center. Cases were selected from our previously reported Penn LBD autopsy cohort⁴ of 133 patients who (1) met clinical criteria for an LBD (PDD or DLB)^{18,19} and (2) had autopsy-confirmed synucleinopathy (ie, brainstem, limbic, or neocortical stage).²⁰ To test clinicopathological associations of dementia in LBDs, we selected the subset of these patients with available antemortem neuropsychological testing data collected after the onset of dementia. Fifty-five LBD (36 PDD, 19 DLB) patients were identified who fulfilled these criteria (Table 1). An age- and sex-matched disease reference cohort of 25 patients with typical amnesic AD and a primary neuropathological diagnosis of AD with an absence of neocortical SYN was selected to examine the distribution of AD pathology in comparison to that seen in LBDs with AD pathology. All autopsies were performed at the Penn Center for Neurodegenerative Disease Research using validated neuropathological criteria²¹ and were analyzed for the presence of copathologies as described.²² All procedures were performed with prior informed consent in accordance with Penn Institutional Review Board guidelines.

Neuropathologic Diagnosis

Fresh tissue samples obtained at autopsy were fixed overnight in 70% ethanol with 150 mM sodium chloride (EtOH) or 10% neutral-buffered formalin (NBF). Tissue samples were processed as described^{17,23} and were embedded into paraffin blocks, and 6 μ m-thick sections were cut for analysis. Sections were stained using immunohistochemistry (IHC) with established antibodies as described.¹⁷ Expert neuropathologists (E.B.L., J.Q.T.) applied current diagnostic criteria to assign Thal phases,²⁴ Braak tau stages,²⁵ CERAD neuritic plaque stages,²⁶ SYN Lewy body (LB) stages,²⁰ and the presence of TDP-43 and aging-related tau astrogliopathy (ARTAG) copathology.²⁷ Final neuropathology diagnosis for each case was rendered using standard semiquantitative assessments for each pathology in each brain region.²¹

Based on modern neuropathological criteria using $A\beta$ amyloid Thal phase, Braak tau stage, and CERAD plaque score (ie, ABC scoring),²¹ we categorized LBD patients into those with a medium or a high level of AD neuropathologic change (ADNPC) sufficient to contribute to dementia²¹ (SYN + AD) and patients with no or low-level AD pathology, who are referred to as those without significant AD copathology (SYN–AD). AD patients were

TABLE 1. Patient Demographics

	LBDs		
	SYN-AD, n = 35	SYN + AD, n = 20	AD, n = 25
Clinical characteristics			
Clinical phenotype	DLB, 7; PDD, 28	DLB, 12; PDD, 8 ^a	AD, 25
Sex, male ^b	26 (74)	15 (75)	15 (60)
Age at onset ^c	61.8 (9.8)	69.0 (6.2) ^a	67.9 (5.7)
Age at dementia ^c	72.5 (6.3)	73.2 (6.7)	67.9 (5.7) ^d
Motor dementia interval ^c	10.8 (7.9)	4.2 (6.2) ^a	NA
Age at death ^c	77.7 (8.7)	78.4 (6.1)	79.4 (6.8)
Disease duration ^c	15.9 (7.2)	9.2 (6.4) ^a	11.52 (5.1)
Neuropathology			
Brain weight ^e	1,276 (260)	1,327 (131)	1,137 (157) ^d
Postmortem interval ^f	13.2 (11.1)	16.0 (8.8)	10.9 (6.3) ^d
McKeith stage ^b			
Brainstem	2 (6)	0 (0)	0 (0)
Limbic	9 (26)	3 (15)	0 (0)
Neocortical	24 (66)	17 (85)	0 (0)
AD level ^b			
None	13 (37)	NA	0 (0)
Low	22 (63)	NA	0 (0)
Medium	NA	12 (60)	2 (8)
High	NA	8 (40)	23 (92)
Other copathology ^b			
PSP	1 (3)	0 (0)	0 (0)
HS	2 (6)	0 (0)	0 (0)
TDP-43	9 (26)	9 (45)	17 (68)
ARTAG	8/26 (31)	13/18 (72) ^a	21 (84)
CVD	0 (3)	1 (5)	2 (8)
AGD	1 (3)	0 (0)	0 (0)

Ten of 25 AD cases had low levels of SYN in amygdala only. TDP-43 pathology was present in hippocampus and/or amygdala.

^a $p < 0.05$ between SYN-AD and SYN + AD.

^bNumber (%); unless specified, all counts are taken from the full group.

^cYears (SD).

^d $p < 0.05$ between SYN + AD and AD

^eGrams (SD).

^fHours (SD).

AD = Alzheimer disease; AGD = argyrophilic grain disease; ARTAG = age-related tau astroglipathy; CVD = cerebrovascular disease; DLB = dementia with Lewy bodies; HS = hippocampal sclerosis; LBD = Lewy body disorder; NA = not applicable; PDD = Parkinson disease with dementia; PSP = progressive supranuclear palsy; SD = standard deviation; SYN = α -synuclein.

similarly assessed by ABC scoring method and additionally were screened for the absence of neocortical SYN.

Digital Pathology

We selected 3 neocortical regions with known domain-specific contributions to cognition in neuropsychological testing for digital analysis, including midfrontal gyrus (MFC), superior temporal gyri (STC), and the angular gyrus (ANG). We also included a limbic region (entorhinal cortex [ERC]) and a subcortical motor region (putamen [PUT]) for comparison. Adjacent sections were immunostained for tau (AT8; Thermo Scientific, Waltham, MA), A β (NAB228; Santa Cruz Biotechnology, Santa Cruz, CA), and SYN (SYN303; Santa Cruz Biotechnology) for use in digital pathology experiments. The majority of slides were fixed in NBF (750/960, 78%), and for those with missing NBF tissue, we used sections from blocks fixed in EtOH. Digital images of histology slides at $\times 20$ magnification were obtained using a Lamina slide scanning system (PerkinElmer, Waltham, MA) and Halo digital image software v1.90 (Indica Labs, Albuquerque, NM) to calculate percentage area occupied (%AO) of reactivity for tau, A β , and SYN pathology as previously published, which included inter-rater validation.²³ Briefly, we used a vertical transect method²³ to sample representative cortical gray matter in neocortical and limbic cortex and used a random sampling from this region of interest for our analyses to reduce sampling bias. Because PUT is a subcortical nucleus without laminar organization, we sampled this region based on microscopic anatomical boundaries of the nucleus. Color deconvolution intensity thresholds were optimized for each stain to detect and quantify the %AO for tau and A β . Because SYN303 IHC can detect nonpathological monomeric SYN in the axon terminal of the neuropil, as well as pathological LBs and Lewy neurites (LNs), we used an additional machine learning step (ie, “classifier” function in HALO) to first segment LB and LN pathology from the background normal neuropil stain based on morphological features prior to applying a color deconvolution algorithm to constrain our detection of pathological SYN in LBs and LNs (Fig 1). We report the average %AO in sampled regions of interest from each slide as we have done previously.²³

Neuropsychological Testing

To test pathological associations with cognitive domains in our LBD dementia cohort, we selected the first available research neuropsychological testing data obtained after the diagnosis of dementia as defined by the diagnostic impression of the clinician from the medical record. Neuropsychological testing was administered to participants by trained research personnel as described.²⁸

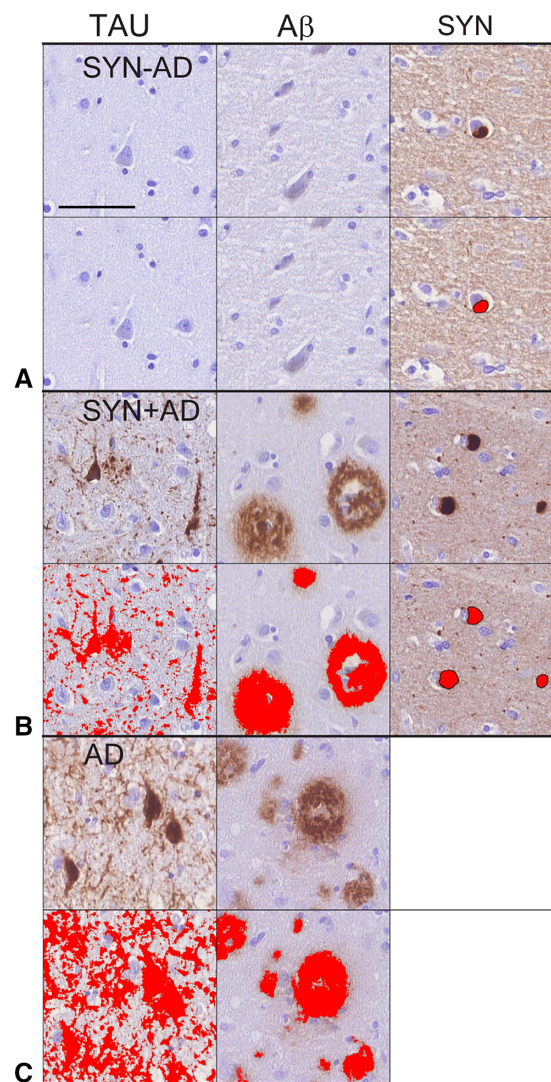


FIGURE 1: Microscopic Pathology of Lewy body disorders and Alzheimer disease (AD). Representative photomicrographs from superior temporal gyri in (A) SYN–AD, (B) SYN + AD, and (C) AD cases stained for tau (AT8, left), β -amyloid (A β , NAB228, middle), and SYN (SYN303, right). The top row for each group shows raw images, and the lower row for each group depicts digital detection of pathology (red overlay indicates percentage area occupied [%AO]). SYN + AD has a higher burden of tau, A β , and SYN pathology compared to SYN–AD, whereas pure AD has much higher cortical tau %AO and similar A β compared to SYN + AD. Images were taken at $\times 32$; scale bar = 100 μ m.

We included neuropsychological tests with sufficient data for analysis, which included 2 tests of global cognition (Mini-Mental State Examination [MMSE] and the Dementia Rating Scale-2 [DRS]), 1 semantic category fluency task (number unique animals named in 60 seconds), and a lexical retrieval task (Boston Naming Task [BNT]).

Statistical Analysis

As %AO data were not normally distributed, a square-root transformation was used for all analyses. %AO

measurements for each stain were compared to ordinal scores (ie, 0 = none, 1 = mild, 2 = moderate, 3 = severe) as done previously²³ as well as neuropathological stages using analysis of variance with post hoc *t* tests. Pathology stage categories were collapsed when a category had <10 patients. Differences in pathological distribution between SYN + AD and SYN-AD groups were assessed using both independent sample *t* tests and analysis of covariance (ANCOVA) models adjusted for age at death, sex, clinical diagnosis (PDD vs DLB), and fixative (NBF vs EtOH).

We performed linear mixed effects models to test the association of pathology group (SYN + AD vs SYN-AD) and neocortical regional burden (ie, MFC, STC, and reference region ANG) for each pathology (A β , tau, SYN) %AO as the dependent variable. The linear mixed-effects model can account for the correlations of pathology measures across regions within each individual. Age at death and sex were included as covariates in these models.

Performance between SYN + AD and SYN-AD groups on individual neuropsychological tests were compared using independent *t* tests. The LBD cohort was also dichotomized by median SYN, tau, and A β AO% measurements to examine digital pathology-defined patient subgroups. Test performance was also directly compared to %AO pathology using partial correlation controlling for age at test or MMSE examining prehypoththesized regions governing specific cognitive tasks (ie, MFC with category fluency, STC with BNT, average cortical pathology with MMSE and DRS).

Differences in pathological distribution of tau and A β between SYN + AD and the reference pure AD group were assessed using *t* tests and ANCOVA adjusting for age at death and sex. We also calculated a ratio of regional tau and A β %AO to the average neocortical tau and A β %AO (eg, region tau %AO/average neocortical tau %AO) for each region and compared ratios between groups to examine the relative neocortical distribution of pathology.

Analyses were performed using SPSS v24 (IBM, Armonk, NY) or Stata v15 (StataCorp, College Station, TX) and were 2-tailed with $\alpha = 0.05$, as we chose specific regions to test clinical-pathological correlations using prespecified hypotheses.

Results

Patients

Characteristics of the LBD and AD patients are described in Table 1. Similar to our previous observations,⁴ patients with SYN + AD pathology were older at onset and had a shorter time interval from onset of motor symptoms to

dementia, reduced survival, and greater frequency of DLB phenotype than PDD (see Table 1). Copathologies other than AD were uncommon in LBDs. More cases with ARTAG were noted in the SYN + AD group than SYN-AD ($\chi^2 = 7.3$, $p \leq 0.007$). There were more cases with limbic TDP in the SYN + AD group as well, but this did not reach significance ($\chi^2 = 2.2$, $p = 0.14$). Patients with limbic TDP-43 pathology tended to have an older age at death ($t_{53} = 2.2$, $p = 0.04$), but this was not observed for patients with ARTAG ($t_{42} = 1.0$, $p = 0.33$).

Digital Measurement of Pathologic Burden in LBDs

There were robust differences across 0 to 3 (ie, none, mild, moderate, severe) ordinal scores for parametric %AO measures of A β ($F_{3, 256} = 380.0$, $p < 0.001$), tau ($F_{3, 256} = 76.1$, $p < 0.001$), and SYN ($F_{3, 257} = 152.0$, $p < 0.001$), suggesting our digital measurements accurately reflect traditional pathology rating scales. Next, we examined the relationship between traditional pathology stages (Thal,²⁴ CERAD,²⁶ Braak tau,²⁵ and McKeith¹⁹) and average neocortical %AO measurements for each respective pathology in LBD cases. There was significant concordance of amyloid Thal phases, CERAD plaque stages, Braak tau stages, and McKeith stages with neocortical average %AO for each respective pathology (Thal phase and A β %AO: $F_{3, 49} = 50.4$, $p < 0.001$; CERAD and A β %AO: $F_{2, 50} = 58.9$, $p < 0.001$; Braak and tau %AO: $F_{2, 52} = 19.4$, $p < 0.001$; McKeith stage and SYN %AO: $F_{1, 53} = 8.3$, $p = 0.006$; Fig 2). Additionally, there was greater average neocortical SYN %AO across the 4 levels of ADNPC ($F_{3, 51} = 5.7$, $p = 0.002$; see Fig 2).

Regional Distribution of Tau, A β , and SYN Pathology in SYN + AD versus SYN-AD LBD Groups

Comparison of SYN pathology between groups revealed higher levels in each individual neocortical region and the average of all neocortical regions in SYN + AD compared to SYN-AD. These differences persisted in multivariate analysis controlling for sex, age at death, fixative, and clinical phenotype (DLB vs PDD; $F_{1, 54} = 5.7-12.2$, $p < 0.001-0.02$). In contrast, SYN %AO burden in the limbic (ERC) and subcortical (PUT) regions was similar levels between the 2 groups (ERC: $t_{47} = 0.5$, $p = 0.6$; PUT: $t_{53} = 1.4$, $p = 0.2$; Table 2, Fig 3).

As expected, regional pathologic burden showed higher tau and A β throughout all brain regions in SYN + AD compared to SYN-AD, which survived correction for sex, age at death, fixative, and clinical phenotype (DLB v PDD) in multivariate analysis (tau: $F_{1, 53} = 12.2$, $p < 0.001$; A β : $F_{1, 53} = 12.2$, $p < 0.001$).

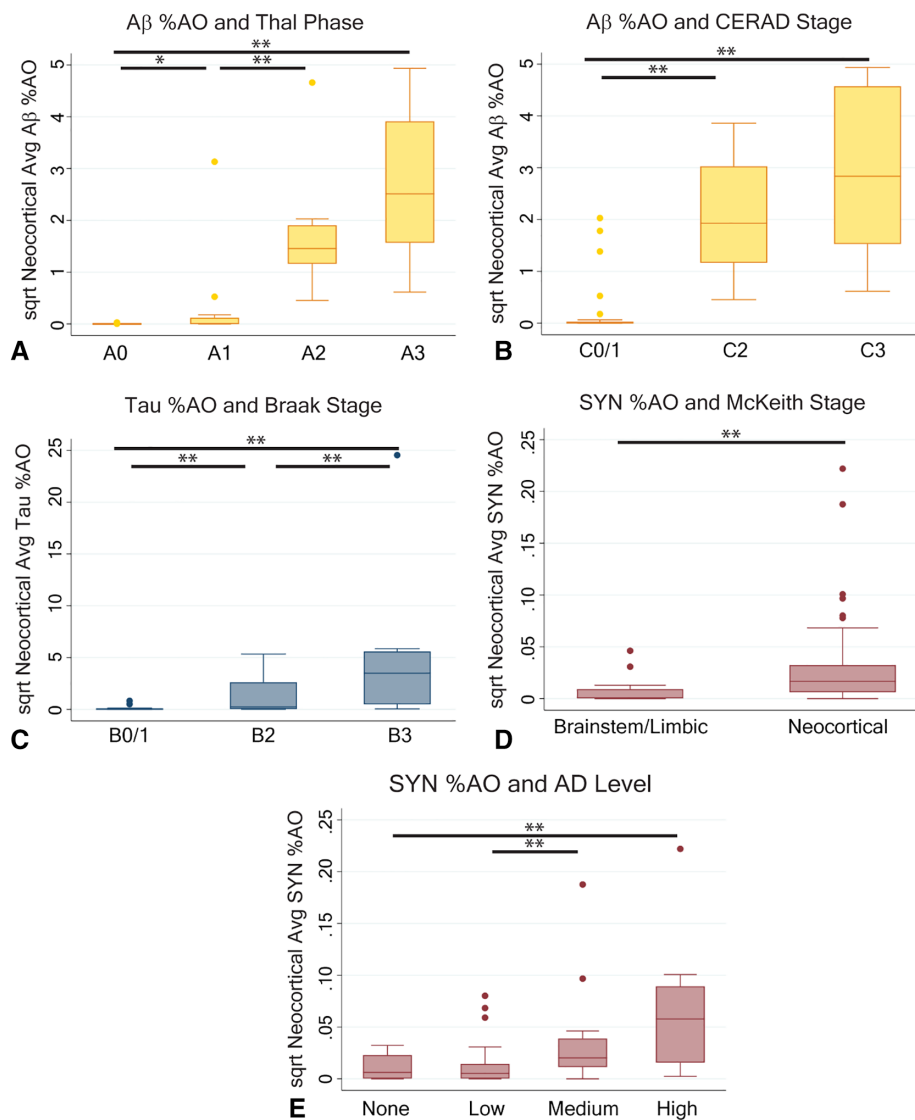


FIGURE 2: Higher pathology stages are associated with higher neocortical percentage area occupied (%AO). Boxplots depict the median, interquartile range, and range of (A) β -amyloid ($A\beta$) %AO in each Thal phase ($F_{49, 3} = 50.4, p < 0.001$), (B) $A\beta$ plaque %AO in each CERAD stage ($F_{50, 2} = 58.9, p < 0.001$), (C) tau pathology %AO in each Braak tau stage ($F_{52, 2} = 19.4, p < 0.001$), (D) α -synuclein (SYN) %AO in each Lewy body disorder stage ($F_{53, 1} = 8.3, p = 0.006$), and (E) neocortical average SYN %AO for each Alzheimer disease (AD) level ($F_{51, 3} = 5.7, p = 0.002$). Increasing stages of pathology are associated with higher measures of neocortical pathology, including increasing SYN pathology for each stage of coexisting AD neuropathologic change. * $p < 0.05$, ** $p < 0.01$ between groups.

$43-54 = 6.3-23.0, p < 0.02$; $A\beta: F_{1, 43-53} = 16.1-46.6, p < 0.001$; see Table 2, Fig 3).

Linear mixed-effect models showed a significant association of SYN + AD with greater overall neocortical $A\beta$ ($\beta = 1.1$, standard error [SE] = 0.09, $t = 12$, $df = 156$, $p < 0.001$), tau ($\beta = 1.1$ SE = 0.13, $t = 8.4$, $df = 159$, $p < 0.001$), and SYN pathology ($\beta = 0.09$, SE = 0.02, $t = 5.3$, $df = 159$, $p < 0.001$).

There was an independent association of region with tau (STC $\beta = 0.3$, SE = 0.2, $df = 159$, $p = 0.03$; in comparison to ANG) and SYN (STC $\beta = 0.1$, SE = 0.02, $df = 159$, $p < 0.01$; MFC $\beta = 0.1$, SE = 0.02, $df = 159$, $p < 0.01$; in comparison to ANG), indicating a

preferential increase in both SYN and tau pathology in the temporal lobe. No regional difference for $A\beta$ was observed ($F = 2.4$, $df = 2, 156$, $p = 0.09$; see Fig 3).

We also tested the relative distribution of SYN in the PUT to the neocortex. A ratio of the average neocortical SYN %AO to PUT SYN %AO showed that SYN + AD had a higher ratio than SYN-AD and therefore relatively greater neocortical burden of SYN pathology ($t_{42} = 2.1, p = 0.04$).

Regional Digital Histology and Cognitive Performance in LBDs

Performance on neuropsychological testing in SYN-AD and SYN + AD showed similar performance on MMSE,

TABLE 2. Pathology Percentage Area Occupied Analysis between SYN–AD, SYN + AD, and Pure AD

Pathology	Region	LBDs		
		SYN–AD, n = 35	SYN + AD, n = 20	AD, n = 25
Tau	MFC	0.16 (0.17), n = 35	0.82 (1.02), n = 20 ^a	6.27 (3.3), n = 25 ^b
	STC	0.22 (0.18), n = 35	1.89 (1.58), n = 20 ^a	7.41 (2.1), n = 25 ^b
	ANG	0.18 (0.21), n = 35	1.06 (1.09), n = 20 ^a	6.25 (2.2), n = 25 ^b
	Neocortical average	0.20 (0.172), n = 35	1.43 (1.14), n = 20 ^a	6.90 (2.1), n = 25 ^b
	ERC	1.59 (1.76), n = 35	4.63 (2.26), n = 20 ^a	7.49 (3.0), n = 25 ^b
	PUT	0.22 (0.16), n = 35	0.62 (0.57), n = 20 ^a	
A β	MFC	0.52 (0.67), n = 35	1.70 (0.54), n = 20 ^a	2.09 (0.67), n = 20 ^c
	STC	0.29 (0.50), n = 34	1.43 (0.46), n = 20 ^a	1.80 (0.61), n = 24 ^c
	ANG	0.29 (0.50), n = 34	1.43 (0.46), n = 20 ^a	1.80 (0.61), n = 24 ^c
	Neocortical average	0.43 (0.61), n = 33	1.57 (0.42), n = 20 ^a	1.92 (0.51), n = 20 ^c
	ERC	0.51 (0.57), n = 33	1.74 (0.60), n = 19 ^a	2.05 (0.60), n = 22 ^c
	PUT	0.46 (0.67), n = 35	1.62 (1.10), n = 20 ^a	
SYN	MFC	0.09 (0.10), n = 35	0.19 (0.18), n = 20 ^a	
	STC	0.09 (0.09), n = 35	0.19 (0.15), n = 20 ^a	
	ANG	0.04 (0.08), n = 35	0.11 (0.12), n = 20 ^d	
	Neocortical average	0.09 (0.08), n = 35	0.19 (0.12), n = 20 ^a	
	ERC	0.12 (0.11), n = 30	0.13 (0.13), n = 19	
	PUT	0.15 (0.13), n = 35	0.24 (0.31), n = 20	

Square root transformed values are reported.

^a $p < 0.01$, SYN–AD vs SYN + AD in univariate analysis.

^b $p < 0.01$, SYN + AD vs AD in univariate analysis.

^c $p < 0.05$, SYN + AD vs AD in univariate analysis.

^d $p < 0.05$, SYN–AD vs SYN + AD in univariate analysis.

AD = Alzheimer disease; ANG = angular gyrus; A β = β -amyloid; ERC = entorhinal cortex; LBD = Lewy body disorder; MFC = midfrontal gyrus; PUT = putamen; STC = superior temporal gyri; SYN = α -synuclein.

DRS, and category fluency. However, SYN + AD patients performed worse on confrontation naming (BNT) than SYN–AD ($t_{27} = 2.1$, $df = 27$, $p = 0.04$; Table 3). There was no difference between SYN + AD and SYN–AD in age at testing, years from dementia onset to testing, years from testing to death, or education level ($p > 0.05$). When the LBD group was divided into high and low tau neuropathology groups based on the median of %AO neocortical tau alone, similar differences between high tau %AO and low tau %AO groups were seen in performance on BNT (see Table 3). No differences in test scores were seen between median divisions of the cohort by %AO of A β or SYN. We also examined LBD patients with or without ARTAG or TDP-43 copathology and similarly did not find significant differences in test scores (data not shown).

In all of the above comparisons, there were no differences in age at test, years from dementia onset to testing, years from testing to death, or education between groups ($p > 0.05$ for each).

Comparing digital measure of pathology directly with test performance showed significant negative correlations of MMSE and DRS with average neocortical tau %AO ($r = -0.45$, -0.68 , $p < 0.001$ for both). For tau %AO pathology in prespecified regions based on known anatomical associations, we found significant negative correlations for category fluency and tau %AO in MFC ($r = -0.44$, $p = 0.005$) and BNT with tau %AO in the STC ($r = -0.39$, $p = 0.04$). There were no significant correlations of test performance with A β or SYN %AO pathology in their corresponding regions of interest

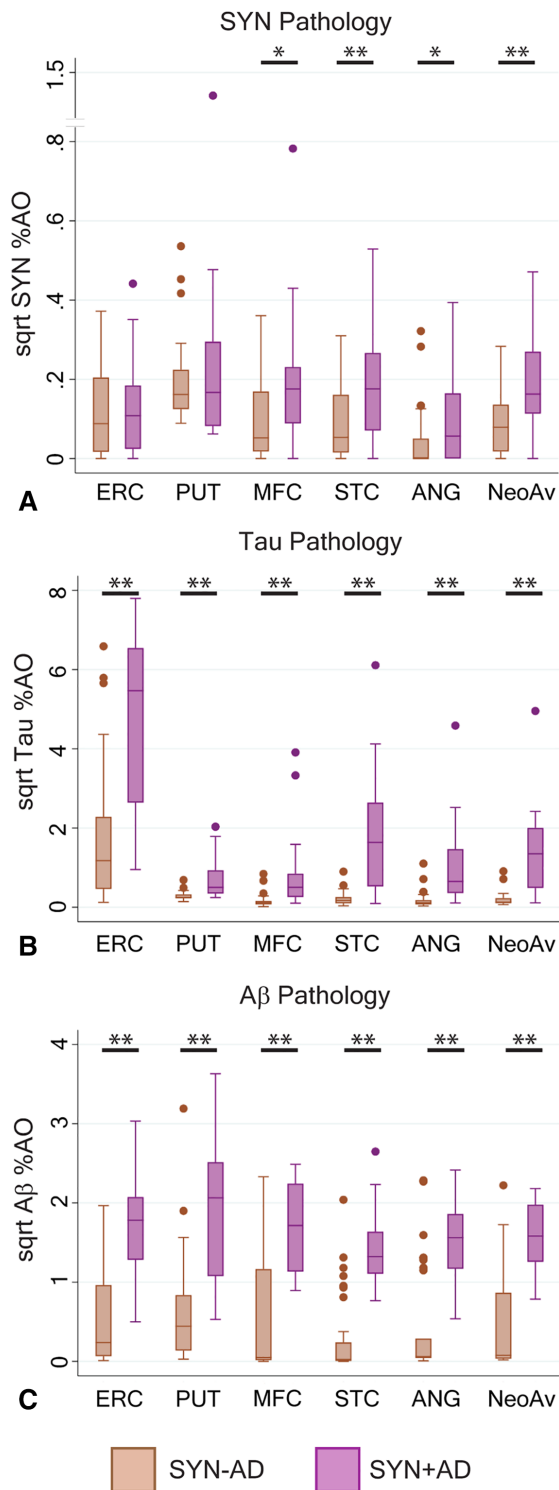


FIGURE 3: Regional pathology in Lewy body disorders with α -synuclein (SYN) + Alzheimer disease (AD) compared to SYN-AD. Boxplots depict median, interquartile range, and range of percentage area occupied (%AO) of (A) SYN pathology, (B) tau pathology, and (C) β -amyloid (A β) pathology in each region and in the average of the 3 neocortical regions. Brown indicates SYN-AD, and purple indicates SYN + AD. * $p < 0.05$, ** $p < 0.01$ in univariate analysis. ANG = angular gyrus; ERC = entorhinal cortex; MFC = midfrontal gyrus; NeoAv = neocortical average; PUT = putamen; STC = superior temporal gyri.

($r = -0.32$ to 0.12 , $p > 0.1$). When covarying for MMSE, there continues to be a significant inverse correlation between neocortical average tau %AO and performance on DRS and with STC tau %AO and BNT ($r = -0.59$ and -0.39 , $p = 0.006$ and 0.04 , respectively); however, category fluency was no longer correlated significantly with MFC tau %AO ($p > 0.1$).

Regional Distribution of Tau and A β in SYN + AD and Pure AD

We compared neocortical and hippocampal tau and A β %AO between the SYN + AD and pure AD groups. Overall, the severity of tau pathology in the AD group was much greater than that seen in the SYN + AD cases for each region examined and in the neocortical average ($t = 5.2-10.5$, $p < 0.001$ for all; Fig 4). These differences persisted when controlling for demographics in multivariate analysis ($F_{1, 44} = 12.9-122.0$, $p < 0.007$). Because the pure AD group is largely high (B3) Braak tau stage (see Table 1), we performed a subgroup analysis to compare SYN + AD with Braak AD tau stage B3 ($n = 10$) to the pure AD group with Braak B3 tau stage ($n = 23$) and still found a higher burden of tau %AO in AD in all neocortical regions and the neocortical average ($t_{31} = 3.2-9.1$, $p < 0.004$ for all) with more similar tau %AO in the ERC ($t_{31} = 1.8$, $p = 0.08$).

We assessed the relative neocortical regional burden of tau %AO in SYN + AD compared to pure AD by comparing ratios of tau %AO in each region to the average neocortical tau %AO (eg, [MFC tau %AO]/[neocortical average tau %AO]). We found pure AD had a relatively greater proportion of tau %AO in MFC compared to SYN + AD ($t_{41} = 3.3$, $p < 0.01$), whereas SYN + AD had a relatively greater proportion of tau %AO in STC compared pure AD ($t_{41} = 2.0$, $p < 0.05$; see Fig 4). SYN + AD and pure AD had similar amounts of A β in the ERC and higher amounts of A β in AD in the MFC, STC, ANG, and neocortical average in univariate analysis; however, these comparisons were not significant in multivariate analysis (for model, $F_{3, 39-43} = 1.6-2.0$, $p > 0.10$; see Table 2). There were no differences in the relative distribution of A β between SYN + AD and AD ($p > 0.05$).

Discussion

Previous postmortem studies using traditional pathologic methods suggest clinically significant AD copathology (ie, medium-high ADNPC) is present in ~50% of all LBDs,^{1,4} is associated with greater overall SYN pathology, and corresponds with decreased survival and faster progression to dementia.^{4,9,10,13,14} This literature suggests that AD copathology plays an integral role in the pathophysiological process of LBDs. Here, using a digital

TABLE 3. Neuropsychological Assessments in SYN + AD versus SYN–AD and Digital Pathology–Defined High versus Low Neocortical Tau Groups in LBDs

Neuropsychological Test	SYN–AD, n = 35	SYN + AD, n = 20	%AO	%AO	%AO	%AO	%AO	%AO
			Low Tau, n = 28	High Tau, n = 27	Low A β , n = 27	High A β , n = 26	Low SYN, n = 28	High SYN, n = 27
MMSE	21.9 (5.1), n = 16	21.5 (7.4), n = 20	22.6 (4.4), n = 28	20.9 (7.2), n = 27	22.5 (4.7), n = 27	21.0 (7.2), n = 26	20.9 (5.9), n = 28	22.7 (5.9), n = 27
Dementia Rating Scale total	114.3 (17.0), n = 16	105.8 (26.9), n = 5	117.1 (11.6), n = 14	102.7 (28.2), n = 7	112.4 (12.1), n = 11	110.3 (26.7), n = 9	107.8 (19.0), n = 12	118.3 (19.2), n = 9
Category fluency	9.6 (4.7), n = 23	9.6 (6.0), n = 18	10.2 (4.8), n = 21	8.9 (5.5), n = 20	10.1 (4.8), n = 16	9.5 (5.4), n = 23	9.3 (5.4), n = 20	9.9 (4.9), n = 21
Boston Naming Test	26.6 (3.4), n = 19	23.1 (5.7), n = 10 ^a	27.0 (3.4), n = 17	23.1 (5.0), n = 12 ^a	26.0 (3.5), n = 14	24.1 (5.5), n = 13	25.0 (5.6), n = 15	25.8 (3.1), n = 14

All data shown are expressed as mean (standard deviation).

^a $p < 0.05$.

MMSE = Mini-Mental State Examination.

histology approach, we found that SYN + AD patients have greater neocortical SYN than SYN–AD patients while having equivalent SYN burden in the ERC and PUT (see Fig 3, Table 2). We also found the topology of neocortical tau pathology in LBDs appears to map more closely to the distribution of SYN pathology (see Fig 3) and diverges from the neocortical pattern of tau pathology in AD (see Fig 4), whereas the diffuse pattern of A β amyloidosis is similar in LBD and AD (see Table 2, Fig 4). Finally, the severity of tau pathology in LBDs correlates with cognitive performance on both global cognitive measures and 2 cortical region–dependent cognitive tasks (see Table 3).

Digital pathology is a novel approach for fine-grained, parametric assessment of disease severity and facilitates improved detection of clinicopathological associations.^{23,29,30} Neuropathological staging systems for LBDs^{20,31} and AD^{24–26} are useful measures of overall disease severity that are reproducible across centers³²; however, they are largely based on the topology of pathology, with less emphasis on severity. We found our digitized measurements of increasing overall neocortical averages of tau, A β , and SYN reflected ordinal stages of pathology for AD and LBD (see Fig 2). These results suggest that digital methods may provide complimentary data to traditional staging schemes for future clinicopathological studies.

When we dichotomized the LBD cohort based on presence or absence of sufficient ADNPC to contribute to dementia,²¹ an increase in neocortical SYN pathology in SYN + AD was noted, with particular increases in the STC and MFC. In contrast, there were equivalent levels of SYN pathology in the PUT and ERC (see Fig 3). The ERC and the PUT are thought to be affected earlier in the spread of SYN in LBDs.^{20,31,33} Thus, one potential interpretation of these results is that spread of SYN pathology to the neocortex in SYN + AD may be facilitated by the pathophysiological processes of tau and A β .

Although tau, A β , and SYN pathology are all correlated in LBDs,^{4,9,10,34} our digitized pathology analysis revealed differences in the regional patterns of pathology. Whereas A β has a relatively diffuse neocortical pattern, tau has a higher concentration in the STC in SYN + AD in a manner that more closely resembles the pattern of neocortical SYN in SYN + AD (see Table 2, Fig 3). Thus, it appears that SYN pathology appears in a distinct distribution in the neocortex when accompanied by tau copathology. Although we cannot rule out that these patterns of tau and SYN pathology in SYN + AD represent in part greater overall severity of pathology, as traditional Braak staging of both SYN^{20,31} and tau²⁵ suggest STC may be affected earlier in the disease compared to other neocortical regions sampled, we do not find a difference in

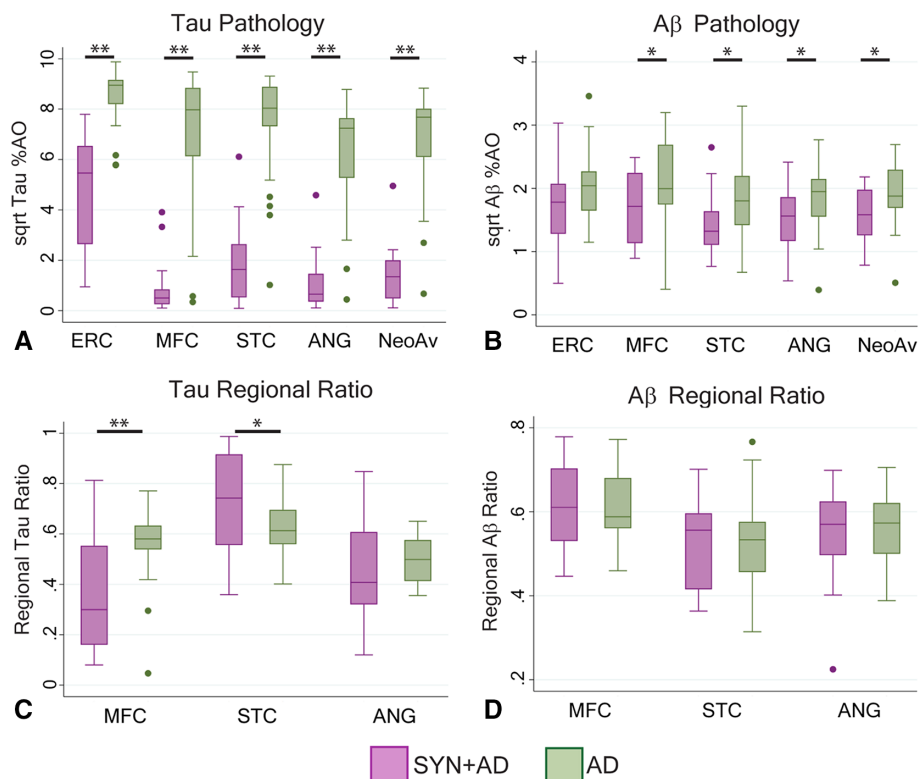


FIGURE 4: Regional tau and β -amyloid ($A\beta$) pathology in Lewy body disorders with α -synuclein (SYN) + Alzheimer disease (AD) compared to AD without neocortical SYN. Boxplots depict median, interquartile range, and range of percentage area occupied (%AO) of (A) tau pathology and (B) $A\beta$ pathology in each region and average of the 3 neocortical regions. Of note, all multivariate models were nonsignificant for $A\beta$ %AO burden in SYN + AD compared to AD, whereas all models and pathology factor variables for tau %AO burden were significant. Boxplots depict the proportion of total pathology in each region (eg, midfrontal gyrus [MFC]/neocortical average [NeoAv]) for (C) tau and (D) $A\beta$. Purple indicates SYN + AD, and green indicates AD. * $p < 0.05$, ** $p < 0.01$ in univariate analysis. ANG = angular gyrus; ERC = entorhinal cortex; STC = superior temporal gyri.

subcortical pathological burden of SYN between the SYN + AD and SYN–AD groups.

Our cohort included both PDD and DLB clinical phenotypes, and we found a greater proportion of clinical DLB patients in the SYN + AD group (see Table 1), consistent with previous studies finding higher neocortical burdens of SYN, tau, and $A\beta$ in DLB compared to PDD^{4,9}; however, we still found a robust difference in the distribution and severity of SYN pathology between SYN + AD and SYN–AD after adjusting for PDD/DLB clinical phenotype (see Table 2), which was not a significant predictor in our analyses (data not shown). Furthermore, although cognitive impairment³⁵ and dementia³⁶ are nearly universal in the long term in LBDs, previous studies suggest a clinico-pathological spectrum,^{4–6} with no clear biological substrate to substantiate the “1-year rule” of dementia onset to distinguish DLB from PDD.^{2,19} As the clinical definitions of LBDs continue to evolve, future efforts should consider clinical and biomarker features predictive of biologically based (ie, pathologic and/or genetic) subgroups of LBDs that may require different treatment strategies.

Our observations suggest that SYN + AD is an important biological subgroup of LBDs with unique clinical

features that are largely driven by tau pathology. AD copathology has been shown to influence gross clinical outcomes,^{4,10,14,22,34} clinical features,^{37–39} and magnetic resonance imaging atrophy patterns.⁴⁰ Here, we examined region-specific associations of pathological burden with cognitive testing in an LBD cohort and found that regional burden of tau is a robust correlate of domain-specific cognitive tasks in LBD (see Table 3). Our direct comparisons found preliminary evidence that SYN + AD performs worse on a naming task reliant on temporal lobe function than SYN–AD, despite a similar level of overall cognitive impairment. Previous work also suggested worse performance in naming tasks^{38,41} in LBDs with AD copathology, and mixed temporal lobe and prefrontal cortex pathology both strongly associated with cognitive decline in LBDs.⁴²

Previous work has highlighted the importance of SYN in the occurrence of dementia and clinical features in LBDs.^{10–12} Here, we focused on LBDs with dementia and did not include nondemented PD patients, which could explain the lack of association of SYN with cognitive measures in this study. Furthermore, it is possible SYN pathology is more influential to early stages of cognitive impairment in LBDs, prior to onset of dementia.

Longitudinal cognitive assessments in autopsy-confirmed cohorts will help resolve these issues. ARTAG was associated with higher ADNPC and TDP-43 was associated with advanced age and higher ADNPC in LBDs, similar to previous reports of these copathologies in AD and aging.²⁷ Although we did not detect an association of these with cognitive scores in this study, we acknowledge that other age-related pathological comorbidities, including cerebrovascular disease, that were less common in our cohort may influence cognition LBDs.

Several strands of evidence suggest a link between tau and SYN pathology in LBDs. Genetic variation in the H1 haplotype of the tau gene *MAPT* has been linked to increased risk for PD⁴³ and DLB,⁴⁴ and the Contursi kindred of hereditary LBD with Ala53Thr mutations in *SNCA* was found to have high levels of tau pathology in addition to SYN.⁴⁵ A subset of tau inclusions in these cases were surrounded by pathological SYN within the same cell.⁴⁵ Moreover, a subset of SYN LBs in sporadic LBDs have peripheral deposits of tau pathology⁴⁶ or tau tangles within the same cell.⁴⁷ There is a large proportion of SYN pathology located at the synapse in LBDs that is visualized only with nontraditional tissue preparations^{48,49} and thus, copolymerization of tau and SYN at the presynaptic compartment may also influence regional spread of tau and SYN pathology. Furthermore, in vitro studies suggest the cocubation of recombinant tau and SYN can accelerate polymerization of both proteins into fibrils consisting largely of homopolymers of either tau or SYN.⁵⁰ A significant proportion of transgenic mice harboring the human Ala53Thr mutation in *SNCA* show tau copathology,⁵⁰ and when these mice are bred to also contain transgenes for human mutations in *APP* and *MAPT*, there is acceleration of cognitive decline and deposition of all 3 pathologies, further suggesting a synergistic interaction of these proteins. Finally, recent data suggest a distinct strain of recombinant SYN that can induce both tau and SYN pathology in cell models¹⁵ that is detected in human LBD brain tissue.¹⁶

Our direct comparisons of SYN + AD and AD suggest that the pattern of tau deposition is different in these 2 conditions. Tau %AO is severalfold higher in AD than SYN + AD, even when comparing patients with similar Braak tau stages. Nevertheless, we found a greater relative pathologic burden of neocortical tau pathology in STC in SYN + AD compared to a greater relative pathologic burden of tau in MFC in pure AD (see Fig 4). It is tempting to hypothesize that the aforementioned strains of pathogenic SYN that cross-seed tau pathology in model systems could contribute to the altered pattern of tau pathology in LBDs observed here.^{15,16} Recently published studies using the positron emission tomography (PET) ligand

flortaucipir similarly found overall lower levels of flortaucipir uptake in LBDs compared to AD patients and increased uptake in the posterior temporal–parietal lobes compared to controls.^{51,52} Although the limited sampling in pathological studies cannot completely recapitulate whole-brain PET imaging analyses, these studies provide converging evidence for a distinct pattern of tau in LBDs. A recent digital pathology study found higher tau pathology in AD compared to SYN + AD in the hippocampus alone.²⁹ This discrepancy may be in part because the previous study largely focused on clinical AD with neocortical SYN pathology, whereas our AD reference cohort was free of neocortical SYN pathology and was age- and sex-matched to our SYN + AD group. Nonetheless, these studies provide complementary views of the clinicopathological spectrum of AD and LBDs, and with the emerging in vivo molecular imaging data, highlight intriguing distinctions of tau pathology in LBDs compared to AD.

There are limitations to the current study. Despite the large-scale digital histology effort (>900 slides digitally analyzed), we sampled limited brain regions in a focused cohort to facilitate correlation with cognitive performance. Future digital pathology studies in larger multicenter cohorts using the full spectrum of LBDs with extensive sampling will be needed to fully elucidate the staging of tau, A β , and SYN in LBDs and compared to AD. Autopsy cohorts from tertiary academic centers may not be completely generalizable to the clinical LBD population; results would benefit from confirmation in population-based cohorts. Finally, this study details the results of harmonized neuropsychological testing across cognitive and movement disorder clinics,²⁸ but despite this significant effort, we had limited clinical data across cognitive domains and lacked a test for episodic memory. Future efforts with harmonized cognitive and motor assessments are needed to fully resolve the clinical phenotype of LBDs with AD copathology.

With these caveats in mind, we conclude that concurrent AD copathology is associated with an altered pattern of SYN deposition in LBDs. Nevertheless, it is the anatomic distribution of tau pathology that appears to be associated with specific antemortem cognitive features in dementia and may contribute to observed clinical heterogeneity of LBDs. Furthermore, the distribution of tau pathology in LBDs may be distinct from that in AD, possibly related to the strain of SYN that also elicits tau copathology only in LBDs. Thus, we contend that SYN + AD is a clinically meaningful subtype of LBD that may be more informative than current clinical distinctions between PDD and DLB; antemortem detection of SYN + AD in LBDs could improve prognostication and may aid in clinical trial stratification for more

homogenous patient populations for both symptomatic and protein-targeted therapies.

Acknowledgment

Research reported in this publication was supported by funding from the NIH National Center for Advancing Translational Sciences (TL1TR001880), National Institute on Aging (AG010124), and National Institute of Neurological Disorders and Stroke (NS088341, NS053488).

We thank C. Zhou, J. Robinson, and T. Shuck for their technical assistance; and the patients and families for their participation in the research.

Author Contributions

D.G.C., C.T.M., D.W., M.G., J.Q.T., and D.J.I. contributed to the conception and design of the study; D.G.C., S.X.X., M.L., A.W., C.P., D.A.W., R.S.A., H.H., H.B.C., R.H., A.S., J.E.D., K.R., E.B.L., V.M.-Y.L., M.G., J.Q.T., and D.J.I. contributed to the acquisition and analysis of data; D.G.C., D.W., C.T.M., D.A.W., R.S.A., A.S., M.G., J.Q.T., and D.J.I. contributed to drafting a significant portion of the manuscript or figures.

Potential Conflicts of Interest

Nothing to report.

References

1. Irwin DJ, Lee VM-Y, Trojanowski JQ. Parkinson's disease dementia: convergence of α -synuclein, tau and amyloid- β pathologies. *Nat Rev Neurosci* 2013;14:626–636.
2. Postuma RB, Berg D, Stern M, et al. MDS clinical diagnostic criteria for Parkinson's disease. *Mov Disord* 2015;30:1591–1601.
3. Boeve BF, Dickson DW, Duda JE, et al. Arguing against the proposed definition changes of PD. *Mov Disord* 2016;31:1619–1622.
4. Irwin DJ, Grossman M, Weintraub D, et al. Neuropathological and genetic correlates of survival and dementia onset in synucleinopathies: a retrospective analysis. *Lancet Neurol* 2017;16:55–65.
5. Ballard C, Ziabreva I, Perry R, et al. Differences in neuropathologic characteristics across the Lewy body dementia spectrum. *Neurology* 2006;67:1931–1934.
6. Lippa C, Duda J, Grossman M, et al. DLB and PDD boundary issues diagnosis, treatment, molecular pathology, and biomarkers. *Neurology* 2007;68:812–819.
7. Goldman JG, Williams-Gray C, Barker RA, et al. The spectrum of cognitive impairment in Lewy body diseases. *Mov Disord* 2014;29:608–621.
8. Jankovic J, McDermott M, Carter J, et al. Variable expression of Parkinson's disease: a base-line analysis of the DATATOP cohort. The Parkinson Study Group. *Neurology* 1990;40:1529–1534.
9. Halliday GM, Holton JL, Revesz T, Dickson DW. Neuropathology underlying clinical variability in patients with synucleinopathies. *Acta Neuropathol* 2011;122:187–204.
10. Irwin DJ, White MT, Toledo JB, et al. Neuropathologic substrates of Parkinson disease dementia. *Ann Neurol* 2012;72:587–598.
11. Hurtig H, Trojanowski J, Galvin J, et al. Alpha-synuclein cortical Lewy bodies correlate with dementia in Parkinson's disease. *Neurology* 2000;54:1916–1921.
12. Graff-Radford J, Aakre J, Savica R, et al. Duration and pathologic correlates of Lewy body disease. *JAMA Neurol* 2017;74:310–315.
13. Jellinger K, Seppi K, Wenning G, Poewe W. Impact of coexistent Alzheimer pathology on the natural history of Parkinson's disease. *J Neural Transm (Vienna)* 2002;109:329–339.
14. Compta Y, Parkkinen L, O'Sullivan SS, et al. Lewy-and Alzheimer-type pathologies in Parkinson's disease dementia: which is more important? *Brain* 2011;134:1493–1505.
15. Guo JL, Covell DJ, Daniels JP, et al. Distinct α -synuclein strains differentially promote tau inclusions in neurons. *Cell* 2013;154:103–117.
16. Covell DJ, Robinson JL, Akhtar RS, et al. Novel conformation-selective alpha-synuclein antibodies raised against different in vitro fibril forms show distinct patterns of Lewy pathology in Parkinson's disease. *Neuropathol Appl Neurobiol* 2017;43:604–620.
17. Toledo JB, Van Deerlin VM, Lee EB, et al. A platform for discovery: the University of Pennsylvania Integrated Neurodegenerative Disease Biobank. *Alzheimers Dement* 2014;10:477.e1–484.e1.
18. Emre M, Aarsland D, Brown R, et al. Clinical diagnostic criteria for dementia associated with Parkinson's disease. *Mov Disord* 2007;22:1689–1707.
19. McKeith IG, Boeve BF, Dickson DW, et al. Diagnosis and management of dementia with Lewy bodies: fourth consensus report of the DLB Consortium. *Neurology* 2017;89:88–100.
20. McKeith IG, Dickson DW, Lowe J, et al. Diagnosis and management of dementia with Lewy bodies: third report of the DLB Consortium. *Neurology* 2005;65:1863–1872.
21. Montine TJ, Phelps CH, Beach TG, et al. National Institute on Aging-Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease: a practical approach. *Acta Neuropathol* 2012;123:1–11.
22. Robinson JL, Lee EB, Xie SX, et al. Neurodegenerative disease comorbid proteinopathies are prevalent, age-related and APOE4-associated. *Brain* 2018;141:2181–2193.
23. Irwin DJ, Byrne MD, McMillan CT, et al. Semi-automated digital image analysis of Pick's disease and TDP-43 proteinopathy. *J Histochem Cytochem* 2016;64:54–66.
24. Thal DR, Rub U, Orantes M, Braak H. Phases of A beta-deposition in the human brain and its relevance for the development of AD. *Neurology* 2002;58:1791–1800.
25. Braak H, Braak E. Neuropathological staging of Alzheimer-related changes. *Acta Neuropathol* 1991;82:239–259.
26. Mirra SS, Heyman A, McKeel D, et al. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part II. Standardization of the neuropathologic assessment of Alzheimer's disease. *Neurology* 1991;41:479–486.
27. Kovacs GG, Robinson JL, Xie SX, et al. Evaluating the patterns of aging-related tau astrogliopathy unravels novel insights into brain aging and neurodegenerative diseases. *J Neuropathol Exp Neurol* 2017;76:270–288.
28. Watson GS, Cholerton BA, Gross RG, et al. Neuropsychologic assessment in collaborative Parkinson's disease research: a proposal from the National Institute of Neurological Disorders and Stroke Morris K. Udall Centers of Excellence for Parkinson's Disease Research at the University of Pennsylvania and the University of Washington. *Alzheimer Dement* 2013;9:609–614.
29. Walker L, McAleese KE, Thomas AJ, et al. Neuropathologically mixed Alzheimer's and Lewy body disease: burden of pathological protein aggregates differs between clinical phenotypes. *Acta Neuropathol* 2015;129:729–748.

30. Neltner JH, Abner EL, Schmitt FA, et al. Digital pathology and image analysis for robust high-throughput quantitative assessment of Alzheimer disease neuropathologic changes. *J Neuropathol Exp Neurol* 2012;71:1075–1085.
31. Braak H, Del Tredici K, Rub U, et al. Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiol Aging* 2003;24:197–211.
32. Montine TJ, Monsell SE, Beach TG, et al. Multisite assessment of NIA-AA guidelines for the neuropathologic evaluation of Alzheimer's disease. *Alzheimers Dement* 2016;12:164–169.
33. Duda JE, Giasson BI, Mabon ME, et al. Novel antibodies to synuclein show abundant striatal pathology in Lewy body diseases. *Ann Neurol* 2002;52:205–210.
34. Halliday G, Hely M, Reid W, Morris J. The progression of pathology in longitudinally followed patients with Parkinson's disease. *Acta Neuropathol* 2008;115:409–415.
35. Pigott K, Rick J, Xie SX, et al. Longitudinal study of normal cognition in Parkinson disease. *Neurology* 2015;85:1276–1282.
36. Kempster PA, O'Sullivan SS, Holton JL, et al. Relationships between age and late progression of Parkinson's disease: a clinico-pathological study. *Brain* 2010;133:1755–1762.
37. Lopez OL, Becker JT, Kaufer DI, et al. Research evaluation and prospective diagnosis of dementia with Lewy bodies. *Arch Neurol* 2002;59:43–46.
38. Peavy GM, Edland SD, Toole BM, et al. Phenotypic differences based on staging of Alzheimer's neuropathology in autopsy-confirmed dementia with Lewy bodies. *Parkinsonism Relat Disord* 2016;31:72–78.
39. Merdes A, Hansen L, Jeste D, et al. Influence of Alzheimer pathology on clinical diagnostic accuracy in dementia with Lewy bodies. *Neurology* 2003;60:1586–1590.
40. McMillan CT, Wolk DA. Presence of cerebral amyloid modulates phenotype and pattern of neurodegeneration in early Parkinson's disease. *J Neurol Neurosurg Psychiatry* 2016;87:1112–1122.
41. Kraybill ML, Larson EB, Tsuang D, et al. Cognitive differences in dementia patients with autopsy-verified AD, Lewy body pathology, or both. *Neurology* 2005;64:2069–2073.
42. Howlett DR, Whitfield D, Johnson M, et al. Regional multiple pathology scores are associated with cognitive decline in Lewy body dementias. *Brain Pathol* 2015;25:401–408.
43. Zabetian CP, Hutter CM, Factor SA, et al. Association analysis of MAPT H1 haplotype and subhaplotypes in Parkinson's disease. *Ann Neurol* 2007;62:137–144.
44. Labbe C, Heckman MG, Lorenzo-Betancor O, et al. MAPT haplotype H1G is associated with increased risk of dementia with Lewy bodies. *Alzheimers Dement* 2016;12:1297–1304.
45. Duda JE, Giasson BI, Mabon ME, et al. Concurrence of alpha-synuclein and tau brain pathology in the Contursi kindred. *Acta Neuropathol* 2002;104:7–11.
46. Ishizawa T, Mattila P, Davies P, et al. Colocalization of tau and alpha-synuclein epitopes in Lewy bodies. *J Neuropathol Exp Neurol* 2003;62:389–397.
47. Schmidt ML, Martin JA, Lee VM-Y, Trojanowski JQ. Convergence of Lewy bodies and neurofibrillary tangles in amygdala neurons of Alzheimer's disease and Lewy body disorders. *Acta Neuropathol* 1996;91:475–481.
48. Colom-Cadena M, Pegueroles J, Herrmann AG, et al. Synaptic phosphorylated α -synuclein in dementia with Lewy bodies. *Brain* 2017;140:3204–3214.
49. Kramer ML, Schulz-Schaeffer WJ. Presynaptic α -synuclein aggregates, not Lewy bodies, cause neurodegeneration in dementia with Lewy bodies. *J Neurosci* 2007;27:1405–1410.
50. Giasson BI, Forman MS, Higuchi M, et al. Initiation and synergistic fibrillization of tau and alpha-synuclein. *Science* 2003;300:636–640.
51. Kantarci K, Lowe VJ, Boeve BF, et al. AV-1451 tau and β -amyloid positron emission tomography imaging in dementia with Lewy bodies. *Ann Neurol* 2017;81:58–67.
52. Gomperts SN, Locascio JJ, Makaretz SJ, et al. Tau positron emission tomographic imaging in the Lewy body diseases. *JAMA Neurol* 2016;73:1334–1341.