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Published in:
Brain

DOI (link to publication from Publisher):
10.1093/brain/awad192

Publication date:
2023

Document Version
Accepted author manuscript, peer reviewed version

Severe cholinergic terminal loss in newly diagnosed dementia with Lewy bodies

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Abstract

Cholinergic changes play a fundamental role in the natural history of Dementia with Lewy bodies and Lewy body disease in general. Despite important achievements in the field of cholinergic research, significant challenges remain. We conducted a study with four main objectives: First, to examine the integrity of cholinergic terminals in newly diagnosed Dementia with Lewy bodies. Second, to disentangle the cholinergic contribution to dementia by comparing cholinergic changes in Lewy body patients with and without dementia. Third, to investigate the *in vivo* relationship between cholinergic terminal loss and atrophy of cholinergic cell clusters in the basal forebrain at different stages of Lewy body disease. Fourth, to test whether any asymmetrical degeneration in cholinergic terminals would correlate with motor dysfunction and hypometabolism.

To achieve these objectives, we conducted a comparative cross-sectional study of 25 newly diagnosed Dementia with Lewy bodies patients (age 74±5 years, 84% male), 15 healthy control subjects (age 75±6 years, 67% male), and 15 Parkinson's disease patients without dementia (age 70±7 years, 60% male). All participants underwent [^{18}F]fluoroetoxybenzovesamicol PET and high-resolution structural MRI. In addition, we collected clinical [^{18}F]fluorodeoxyglucose PET images. Brain images were normalized to standard space and regional tracer uptake and volumetric indices of basal forebrain degeneration were extracted.

Patients with dementia showed spatially distinct reductions in cholinergic terminals across the cerebral cortex, limbic system, thalamus, and brainstem. Also, cholinergic terminal binding in

cortical and limbic regions correlated quantitatively and spatially with atrophy of the basal forebrain. By contrast, patients without dementia showed decreased cholinergic terminal binding in the cerebral cortex despite preserved basal forebrain volumes. In patients with dementia, cholinergic terminal reductions were most severe in limbic regions and least severe in occipital regions compared to those without dementia. Interhemispheric asymmetry of cholinergic terminals correlated with asymmetry of brain metabolism and lateralized motor function.

In conclusion, this study provides robust evidence for severe cholinergic terminal loss in newly diagnosed Dementia with Lewy bodies, which correlates with structural imaging measures of cholinergic basal forebrain degeneration. In patients without dementia, our findings suggest that loss of cholinergic terminal function occurs *before* neuronal cell degeneration. Moreover, the study supports that degeneration of the cholinergic system is important for brain metabolism and may be linked with degeneration in other transmitter systems. Our findings have implications for understanding how cholinergic system pathology contributes to the clinical features of Lewy body disease, changes in brain metabolism, and disease progression patterns.

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Running title: Cholinergic loss in Lewy body disease

Keywords: cholinergic neurons; fluoroetoxybenzovesamicol; VAChT Proteins; functional laterality; neurodegenerative diseases; Lewy body dementia

Abbreviations: FDG = fluorodeoxyglucose; FEOBV = fluoroetoxybenzovesamicol; MoCA = Montreal Cognitive Assessment; RBDSQ = REM Sleep Behavior Disorder Screening Questionnaire; UPDRS-III = Unified Parkinson's disease rating scale, part III

Introduction

Dementia with Lewy bodies is characterized by pathological changes in cholinergic neurons in manifest and prodromal disease stages.[1-7] Also, cholinergic changes have been observed in non-demented stages of Lewy body disease, such as isolated REM sleep behavior disorder and Parkinson's disease.[6,8] As such, studying cholinergic changes is critical for advancing our understanding of Dementia with Lewy bodies and Lewy body diseases in general.

This study addresses four important aspects that need further investigation. First, cholinergic terminals can be visualized with [^{18}F]fluoroetoxybenzovesamicol ([^{18}F]FEOBV) PET. The tracer targets the vesicular acetylcholine transporter and is currently the most specific *in vivo* marker of cholinergic terminals. While two promising preliminary studies have been conducted using

[^{18}F]FEOBV PET in small samples of patients with manifest Dementia with Lewy bodies, these results need to be confirmed in larger and more recently diagnosed patient samples.[9,10]

Second, the cholinergic contribution to the clinical and cognitive features of Lewy body disease is not fully understood. This contribution may be clarified by investigating thoroughly clinically characterized Dementia with Lewy bodies patients with [^{18}F]FEOBV PET. Also, more than 80% of patients with Parkinson's disease develop Parkinson's disease dementia, and the cholinergic changes in Parkinson's disease dementia and Dementia with Lewy bodies are nearly identical.[4,5,11,12] Therefore, it may be possible to delineate the cholinergic contribution to dementia by comparing Dementia with Lewy bodies patients and Parkinson's disease patients without dementia.

Third, degeneration of cortically-projecting cholinergic cell clusters in the basal forebrain is a well-established pathological feature of Lewy body disease that can be measured indirectly through basal forebrain volume reductions on structural MRI scans.[13,14] Comparing basal forebrain atrophy and cortical cholinergic terminal loss in Dementia with Lewy bodies and Parkinson's disease may reveal novel insights into how these two markers of neurodegeneration progress over the disease course and whether they are coupled or uncoupled.

Fourth, asymmetry in Lewy body disease may not be limited to the dopamine system but also involve more widespread regions of the CNS.[15] According to the recently proposed *synuclein, origin, and connectome* (SOC) model, understanding asymmetry may be important to explain how Lewy body disorders develop and progress.[16] It remains to be tested whether any asymmetrical degeneration in cholinergic terminals would correlate with motor dysfunction and with hypometabolism as measured with [^{18}F]fluorodeoxyglucose ([^{18}F]FDG) PET.

Thus, we aimed to (i) examine the integrity of cholinergic terminals in Lewy body patients with [^{18}F]FEOBV PET, (ii) measure basal forebrain volumes with MRI, (iii) compare findings in Dementia with Lewy bodies and Parkinson's disease without dementia, and (iv) correlate

asymmetry in [^{18}F]FEOBV PET, motor dysfunction and [^{18}F]FDG PET.

Materials and methods

Setting and participants

This comparative cross-sectional study was conducted at the Department of Nuclear Medicine and PET, Aarhus University Hospital, Denmark, between 2020 and 2022. Patients with Dementia with Lewy bodies (*n*=25, age 74±5 years, 84% male) were recruited from clinical departments and through advertisements. All patients were diagnosed by neurologists according to current diagnostic criteria.[17] The standardized criteria categorize patients as either 'probable' or 'possible' based on the presence of specific clinical features and biomarkers, reflecting the overall level of diagnostic certainty. To enhance the generalizability of our study, we aimed to recruit a large and representative sample of patients from various centers. Inclusion criteria were a Dementia with Lewy bodies diagnosis with generalized low uptake and reduced occipital activity on [^{18}F]fluorodeoxyglucose ([^{18}F]FDG) PET or reduced dopamine transporter uptake in the basal ganglia demonstrated by PET or single-photon emission computed tomography (SPECT). Exclusion criteria were diabetes, schizophrenia spectrum disorder, bipolar disorder, stroke, brain tumor, and alcohol abuse; contraindications to MRI; and previous surgery or radiation therapy to the brain. Cognitively intact elderly control subjects (*n*=15, age 75±6 years, 67% male) were recruited through local newspaper advertisements with similar exclusion criteria and matched to the Dementia with Lewy bodies patients based on sex and age. Healthy control subjects with a score below 26 on the Montreal Cognitive Assessment (MoCA) were excluded.[18] Reasons for non-participation are presented in the Supplementary. We further included a sample of Parkinson's disease patients without dementia (*n*=15, age 70±7 years, 60% male) who were investigated with identical [^{18}F]FEOBV PET protocols.[8] The study was conducted according to the Declaration of Helsinki and approved by the Regional Ethics Committee (project number 1-10-72-270-19). All participants provided written informed consent.

Image acquisition

PET was performed on a Siemens Biograph Vision 600 PET/CT camera 180 – 210 minutes after injection of ~200 MBq [^{18}F]FEOBV. A CT scan was performed for attenuation correction. Medications were not paused prior to the PET examination. In addition to the [^{18}F]FEOBV PET images, we also collected clinical [^{18}F]FDG PET volumes on the Dementia with Lewy bodies patients. These [^{18}F]FDG volumes were obtained from different clinical departments using various PET scanners and protocols. MRI was obtained on a 3T Siemens Magnetom Skyra. We used T1 MPRAGE scans for coregistration and volumetric analyses.

Image processing

[^{18}F]FEOBV PET image volumes were binned into six frames of 5 minutes and reconstructed with resolution recovery and time-of-flight, eight iterations, five subsets, 440 matrix, zoom 2, no filter, with a final voxel size of 0.83x0.83x1.65 mm^3 and a spatial resolution of 2 mm full-width half-maximum. Frames were adjusted for head motion, averaged, and co-registered with the T1 images. The T1 images were then segmented and normalized to the MNI152NLin2009cAsym template in SPM12 with a high-dimensional registration algorithm.[19] The resulting deformation fields were used to normalize PET images to MNI standard space. PET images were normalized to patient weight and injected dose to calculate standard uptake values. An analogous approach was followed for the clinical [^{18}F]FDG PET images. Then, [^{18}F]FEOBV PET was intensity-normalized to the centrum semiovale and [^{18}F]FDG PET to cerebellar uptake to yield standard uptake value ratios (SUVR).[10] Specific binding ratios (SBR) for [^{18}F]FEOBV were approximated as SUVR–1. Regional differences were calculated in absolute terms (SBR^2–SBR^1) and relative binding terms ((SBR^2–SBR^1)/SBR^1). Regional tracer uptake was quantified by superimposing stereotactic MNI-space atlases to the PET images.[20-31] Of note, Yeo's atlas is a set of seven anatomical volumes of interest representing distinct cortical networks based on functionally coupled regions across the cerebral cortex.[27] Correction for partial volume effects was performed using the Müller-Gärtner method.[32] Images with and without partial volumes correction were compared. PET images for voxel-wise analyses were smoothed with a Gaussian kernel of 10x10x10 mm.

Volumes of the anterior and posterior cholinergic basal forebrain were extracted from the structural MRI scans using an established automated volumetry approach in combination with a stereotactic atlas of the cholinergic forebrain nuclei in MNI space.[30] This atlas divides the cholinergic basal forebrain into functionally-defined anterior and posterior subdivisions, which largely correspond to cytoarchitectonically-defined boundaries of the medial septum/diagonal band (Ch1-3) and the nucleus basalis Meynert (Ch4), respectively. The resulting basal forebrain volumes were corrected for total intracranial volumes defined as the sum of grey matter, white matter, and cerebrospinal fluid. Image processing was performed in PMOD and the CAT12 toolbox in SPM, and partial volume correction with the PETPVE12 toolbox in SPM.

Clinical assessment

Core features of Dementia with Lewy bodies were assessed with the Mayo Fluctuations Scale, REM Sleep Behavior Disorder Screening Questionnaire (RBDSQ), North-Eastern Visual Hallucinations Interview, and MDS-UPDRS Part III (UPDRS-III).[33-36] We defined fluctuations as a score above 2 on the Mayo Fluctuations Scale, and probable REM sleep behavior disorder as a score above 5 on the RBDSQ. We evaluated hallucinations on the North-Eastern Visual Hallucinations Interview (yes/no). Motor signs were quantified with the Alternate Tapping Test in which participants were instructed to alternately tap with the index finger each of two 1.5 cm wide stickers located 20 cm apart.[37] Odor identification was quantified with Sniffin' Sticks 16-item smell test, and color vision with the FM-100 Color Vision test.[38,39] Depressive symptoms were assessed with the 15-item Geriatric Depression Scale and depression defined as a score above 5.[40] Finally, blood pressure was measured after 15 minutes of supine rest, and then after one, two, and three minutes in the orthostatic position. Clinical assessments for all participants were performed on the same day as [^{18}F]FEOBV PET. Patients with Dementia with Lewy bodies were tested *on*, and patients with Parkinson's disease *off*, dopaminergic medication.

Cognitive assessment

Memory function was assessed using the Hopkins Verbal Learning Test – Revised and Location Learning Test.[41,42] Attention was measured with the Letter-Number Sequencing task, and

executive function was evaluated using Phonemic Verbal Fluency, Alternating Verbal Fluency, and the Wisconsin Cart Sorting test.[43] Language skills were assessed with the 30-item Boston Naming test and Semantic Verbal Fluency, and visuospatial function was evaluated using the Royal Clox, Benton Judgement of Line Orientation, Visual Object and Space Perception, and Pareidolia test.[44-47] Global cognitive function was screened with the MoCA, and premorbid function was measured with the Danish Adult Reading Test.[48] Total scores and sub-scores were calculated as described in the relevant manuals.

[^{18}F]FEOBV PET uptake across groups

We compared [^{18}F]FEOBV uptake in Dementia with Lewy bodies patients to that in healthy control subjects and Parkinson's disease patients using both regional and voxel-wise analysis. Regional differences were calculated in absolute terms, relative terms, and effect size. Receiver operating characteristic (ROC) curves were explored for anatomical regions with large group differences in [^{18}F]FEOBV uptake between Dementia with Lewy bodies patients and healthy controls. We used voxel-wise analyses to investigate effects of age and smoking on [^{18}F]FEOBV uptake in Dementia with Lewy body patients. Additionally, we performed regional analyses to compare subgroups according to medication status and diagnostic certainty.

Associations of clinical features with [^{18}F]FEOBV PET uptake in Dementia with Lewy bodies patients

We used voxel-wise t-tests to compare [^{18}F]FEOBV uptake in Dementia with Lewy bodies patients with and without hallucinations, and in patients with and without fluctuations. We used voxel-wise regression to correlate [^{18}F]FEOBV uptake with the UPDRS-III score. In addition, we correlated color vision with [^{18}F]FEOBV uptake in the lateral geniculate, superior colliculus, and cortical visual network. Odor identification was correlated with [^{18}F]FEOBV uptake in the olfactory cortex.

Associations of cognitive function with [^{18}F]FEOBV PET uptake in

Dementia with Lewy bodies patients

We used voxel-wise regression analysis to examine the effects of composite cognitive z-scores on [^{18}F]FEOBV uptake in Dementia with Lewy bodies patients. Cognitive z-scores were calculated for each test using the healthy control scores as a reference. For non-normally distributed test scores, we calculated modified z-scores by first determining the median test score in the healthy control group ($\bar{x}$), and the absolute difference from the median for each datapoint (x_i-$\bar{x}$). We then calculated the median absolute deviation (MAD) and mean absolute deviation (MeanAD) as the median or mean of the absolute differences in the healthy control group.

$$\frac{0.6745(x_i\text{-}\bar{x})}{\text{MAD}} \text{ if MAD}\neq 0 \qquad (1)$$

and

$$\frac{0.7979(x_i\text{-}\bar{x})}{\text{MeanAD}} \text{ if MAD}=0 \qquad (2)$$

We calculated composite z-scores within domains as the median of the individual test z-scores, and a global combined z-score as the median of composite z-scores. Time between [^{18}F]FEOBV PET and cognitive examinations was 1.14 (5.14) weeks for Dementia with Lewy bodies patients and 1.57 (8.71) weeks for healthy controls.

Basal forebrain volumes and [^{18}F]FEOBV PET

We compared anterior and posterior basal forebrain volumes in Dementia with Lewy bodies patients, Parkinson's disease patients, and healthy control subjects. Associations of basal forebrain volumes with [^{18}F]FEOBV uptake were investigated with voxel-wise regression. In additional region-wise analyses, the anterior basal forebrain volume was correlated with regional [^{18}F]FEOBV uptake in the hippocampus and olfactory cortex, and the posterior basal forebrain volume was correlated with [^{18}F]FEOBV uptake in the global cortex. These correlations were performed separately in Dementia with Lewy bodies patients, Parkinson's disease patients, and

healthy controls, as well as in a combined group of Lewy body patients (Dementia with Lewy bodies and Parkinson's disease).

Asymmetry in [^{18}F]FDG PET, motor function and [^{18}F]FEOBV PET

Asymmetry indices for global cortical interhemispheric [^{18}F]FDG and [^{18}F]FEOBV PET uptake were calculated as:

$$\frac{right - left}{(right + left)/2} \quad (3)$$

Similar asymmetry indices were calculated for the UPDRS-III extremity scores and the Alternate Tapping Test and then correlated with the PET asymmetry indices in the Dementia with Lewy bodies and healthy control groups.

In general, voxel-wise group comparisons were performed with pairwise two-sample t-tests, and voxel-wise correlations with regression analyses. Voxel-wise results were first explored with a voxel-level threshold of $P<0.05$, FDR corrected. If no significant clusters appeared, we explored more lenient uncorrected thresholds of $P<0.001$ or $P<0.01$. All voxel-wise analyses were based on a minimum cluster size of 50 voxels. Distribution was explored with histograms and QQ-plots. For paired and unpaired group comparisons of continuous variables, we used paired or unpaired t-tests for parametric data, or Wilcoxon matched-pairs signed rank or Mann-Whitney tests for non-parametric data. Categorical variables were compared with Fisher's exact test. Effect size was quantified with Cohen's *d*. Correlations were calculated using Spearman's rank correlation or Pearson correlation, as appropriate. Results of multiple group comparisons or multiple correlations were considered significant after FDR-correction. The data set was practically complete and missing clinical or cognitive observations (0.5%) were simply discarded. We used STATA 17 for analyses of clinical variables and GraphPad Prism 9 for

graphical data presentation.

Data availability

Data sharing would require a formal data sharing agreement approved by the relevant local ethics committees.

Results

Participant demographics and clinical assessments

Demographic and clinical data of the Dementia with Lewy bodies, Parkinson's disease, and healthy control groups are presented in Table 1. Compared to healthy controls, Dementia with Lewy bodies patients had more motor and non-motor symptoms and signs, lower performance on motor and non-motor tests, and more cognitive impairment in all domains (Table 1 and Table 2). Compared to Parkinson's disease patients with similar age and sex composition and median disease duration of 8 years, newly diagnosed Dementia with Lewy bodies patients had higher RBDSQ-score, more possible REM sleep behavior disorder, reduced odor identification, increased blood pressure dynamics, and non-significantly higher UPDRS-III score. In the Alternate Tapping Test, healthy control subjects performed better with the right hand compared to the left, P=0.0114, paired t-test. Conversely, right-handed Dementia with Lewy bodies patients performed no better with their right compared to left hand, P=0.1445, paired t-test. In neuropsychological testing, Dementia with Lewy bodies patients were most affected in the attention domain followed by the executive and visuospatial domains. In addition, Dementia with Lewy bodies patients had lower premorbid function compared to healthy controls. Premorbid function did not correlate with cognitive domain scores in the Dementia with Lewy bodies group.

[^{18}F]FEOBV PET uptake in Dementia with Lewy bodies and group comparisons

Table 3 presents the regional [^{18}F]FEOBV PET uptake with relative differences and statistical comparisons between groups. Supplementary Table 1 presents an expanded version of Table 3 including additional nuclei of the thalamus and brainstem. Across all three groups, highest levels of tracer binding were observed in the putamen, caudate, and accumbens, as well as the centromedian and lateral geniculate thalamic nuclei, and the anterior and posterior sectors of the basal forebrain. Intermediate binding was detected in most investigated nuclei of the thalamus, hypothalamus, and brainstem, as well as the cerebellar vermis and flocculus, and limbic regions of the cortex including the hippocampus, amygdala, and insula. Lowest levels of binding were found in the cerebellar lobes and non-limbic areas of the cerebral cortex (Fig. 1).

Highest effect sizes of reduced [^{18}F]FEOBV uptake in Dementia with Lewy bodies patients compared to healthy control subjects were observed in Heschl's gyrus (d=3.00, -85% relative reduction), somatomotor network (2.99, -72%), and the parietal lobe (2.76, -64%) (Fig. 2A, top row). Area under the ROC curve for separating Dementia with Lewy bodies patients from healthy controls was 0.98, 95% confidence interval [0.94; 1.00] for Heschl's gyrus, 0.98 [0.94; 1.00] for the somatomotor network, and 0.97 [0.91; 1.00] for the parietal lobe (all significant at P<0.0001). We found no significant differences in cerebellar or hypothalamic subregions.

Voxel-wise comparison of [^{18}F]FEOBV uptake in healthy control subjects and Dementia with Lewy bodies patients showed one confluent cluster encompassing the entire neocortex thresholded at P<0.05, FDR-corrected. The largest differences were found in the primary visual cortex, posterior cingulate/precuneus, superior temporal areas, and dorsolateral prefrontal cortex (Fig. 2B, top).

Probable Dementia with Lewy bodies patients (n=21) had lower [^{18}F]FEOBV uptake in the temporal cortex (P=0.021), occipital cortex (P=0.028), and thalamus (P=0.019) compared to possible Dementia with Lewy bodies patients (n=4). We found no regions with higher [^{18}F]FEOBV uptake in patients with probable compared to possible Dementia with Lewy bodies. Age and smoking had no effects on [^{18}F]FEOBV uptake in Dementia with Lewy bodies patients in voxel-wise regression analyses at a lenient threshold of P<0.001, uncorrected. Dementia with Lewy bodies patients taking acetylcholinesterase inhibitors (n=21) did not differ from those not

taking the medication (n=4) in terms of age, time since diagnosis, or regional [^{18}F]FEOBV uptake. The same was true for patients taking levodopa medication (n=8) compared to those who did not (n=17).

In Dementia with Lewy bodies compared to Parkinson's disease patients, we found pronounced regional differences in several limbic areas, the habenula and medial geniculate nucleus of the thalamus, and the cerebral cortex except posterior regions. High effect sizes were observed in the hippocampus (d=1.83, -20% relative reduction), olfactory cortex (1.73, -20%), and limbic network (1.70, -26%) (Fig. 2A, middle row). Small effect sizes were found in the occipital lobe (0.05, +3%), visual network (0.29, -20%), and cerebellum (0.24, -7%) (Fig. 2A, bottom row). There were no regions in Parkinson's disease patients with lower [^{18}F]FEOBV PET uptake compared to Dementia with Lewy bodies patients. Results of the voxel-wise analysis aligned well with the regional differences (Fig. 2B, bottom).

Partial volume correction with the Müller-Gärtner method increased the relative regional differences between groups and the variance in data points. Partial volume correction did not change the group-wise statistical comparisons in any cortical region from significant to non-significant or vice-versa. We came to similar conclusions when performing partial volume correction using the geometric transfer matrix method and when comparing subcortical regions.[49] Also, there were no discernable changes in voxel-wise analyses performed with and without partial volume correction. Therefore, we decided to present all data without partial volume correction. Examples of data with and without partial volume correction are presented in Supplementary Table 2 and Supplementary Fig. 1.

Associations of clinical features with [^{18}F]FEOBV PET uptake in Dementia with Lewy bodies patients

In voxel-wise tests, even at a relatively lenient thresholds of P<0.001, uncorrected, no significant associations with [^{18}F]FEOBV uptake were observed for presence of hallucinations or fluctuations, nor for UPDRS-III score, performance on color vision, or odor identification tasks. Complementary regional analysis for specific brain regions typically associated with the

different symptoms (e.g. cortical visual network for color vision, and olfactory cortex for odor identification) also did not yield any significant associations.

Associations of cognitive function with [^{18}F]FEOBV PET uptake in Dementia with Lewy bodies patients

Voxel-wise analyses of [^{18}F]FEOBV uptake with the memory domain composite z-score as a regressor yielded one cluster in the left hippocampus (T=4.28, P<0.001, uncorrected). The attentional domain composite z-score correlated with [^{18}F]FEOBV uptake in the left dorsal posterior cingulate cortex (T=3.25, P<0.01, uncorrected). The executive domain composite z-score correlated with tracer uptake in the left entorhinal cortex (T=3.33) and in the right basal forebrain (T=3.18), both thresholded at P<0.01, uncorrected. The language and visuospatial domains, and the global MoCA score and combined domain z-score showed no effects on [^{18}F]FEOBV uptake.

Cholinergic basal forebrain degeneration and its association with [^{18}F]FEOBV PET uptake

Dementia with Lewy bodies patients had lower anterior (-6%, P=0.0029) and posterior (-16%, P<0.0001) basal forebrain volumes compared to healthy control subjects (Fig. 3B). Also, Dementia with Lewy bodies patients had lower anterior (-9%, P=0.015) and posterior (-13%, P=0.0001) basal forebrain volumes compared to Parkinson's disease patients. Basal forebrain volumes were not different in Parkinson's disease compared to healthy controls. In Dementia with Lewy bodies patients, anterior basal forebrain volumes correlated with [^{18}F]FEOBV uptake in the hippocampus and olfactory cortex, and posterior basal forebrain volumes correlated with global cortical [^{18}F]FEOBV uptake (Fig. 3C). Similar but stronger correlations were found in the combined group of Lewy body patients (Dementia with Lewy bodies and Parkinson's disease). However, we found no significant correlations in the Parkinson's disease group separately or in the healthy control subjects. Full results of association analyses between basal forebrain volumes and regional [^{18}F]FEOBV PET uptake across the different groups are presented in

Supplementary Table 3.

Complementary voxel-wise regression analyses of [^{18}F]FEOBV uptake with anterior basal forebrain volume as a regressor confirmed several clusters of significant associations in Dementia with Lewy bodies patients, most notably in the parahippocampal gyrus, superior temporal areas covering Heschl's gyrus, the anterior cingulate cortex, and prefrontal areas (Fig. 3D, top). Analogous analysis with the posterior basal forebrain volume as regressor showed a comparable but more pronounced pattern of significant associations with cortical [^{18}F]FEOBV uptake, further extending into the motor and supplementary motor cortex and the insula (Fig. 3D, bottom).

Asymmetry in [^{18}F]FEOBV PET, motor function, and [^{18}F]FDG PET

The asymmetry index of the Alternate Tapping Test correlated with the asymmetry index of global cortical [^{18}F]FEOBV PET in Dementia with Lewy bodies patients (Fig. 4A), but not in healthy control subjects. Asymmetry of motor signs measured with the UPDRS-III did not correlate with [^{18}F]FEOBV PET in either Dementia with Lewy bodies patients or healthy controls.

In Dementia with Lewy bodies patients, the global cortical asymmetry index of [^{18}F]FDG PET correlated with the global cortical asymmetry index of [^{18}F]FEOBV PET (Fig. 4B). These [^{18}F]FDG images were collected on 23 (92%) Dementia with Lewy bodies patients, obtained on different locations and with different PET scanners. Because of heterogeneity in the [^{18}F]FDG PET images, and lack of an FDG comparator group, we used these [^{18}F]FDG images only to test this simple measure of global asymmetry, which is less affected by inter-scanner variation.

Post-hoc analyses

We repeated the main analyses excluding the four patients with possible Dementia with Lewy bodies. The exclusion did not significantly affect the regional analyses, voxel-wise analyses, or correlational analyses, except for one specific correlation between posterior basal forebrain volume and global cortical [^{18}F]FEOBV uptake, which became non-significant after excluding

the four patients (data not shown).

Discussion

We found substantial and extensive reductions of cholinergic terminals in a well-characterized group of newly diagnosed Dementia with Lewy bodies patients using [^{18}F]FEOBV PET. The cholinergic denervation was distinct and heterogeneous across the cerebral cortex, limbic system, thalamus, and brainstem. The differences in [^{18}F]FEOBV uptake between Dementia with Lewy bodies and Parkinson's disease patients were most pronounced in limbic regions and least pronounced in occipital regions. Cholinergic terminal binding in limbic and cortical regions correlated quantitively and spatially with subregional basal forebrain atrophy in Dementia with Lewy bodies patients, but not in Parkinson's disease patients. In Dementia with Lewy bodies patients, the interhemispheric distribution of cholinergic terminals correlated with brain metabolism and lateralized motor function.

Severe cholinergic terminal loss in newly diagnosed patients with Dementia with Lewy bodies

The first [^{18}F]FEOBV PET study in four Dementia with Lewy bodies patients reported uptake in the cortical lobes, hippocampus, amygdala, and thalamus.[10] Despite a mean disease duration of 4.2±2.2 years in the patients of that study, the regional uptake values were very similar to those presented in our current study of newly diagnosed Dementia with Lewy bodies patients. The second [^{18}F]FEOBV PET study in five Dementia with Lewy bodies identified clusters of reduced uptake in regions of the insula, cingulum, thalamus, and hippocampus.[9] As such, our results confirm and extend previous findings to a sizeable group of newly diagnosed Dementia with Lewy bodies patients.[50]

We identified significant group differences in multiple brain regions, with limited overlap between healthy controls and Dementia with Lewy bodies patients. Although not designed to evaluate diagnostic accuracy, our ROC analyses demonstrated excellent discriminative ability. Consistent with our findings, other research suggests that [^{18}F]FEOBV PET is sensitive for

distinguishing patients with Alzheimer's disease from healthy controls.[51] Together, these results strengthen the prospects of [^{18}F]FEOBV PET in cholinergic research. In our study, the cerebellum generally showed the smallest group differences (Supplementary Table 1). Accordingly, the uptake in the vestibular nuclei complex, which projects cholinergic fibers to the cerebellum,[52] was not significantly different between the groups.

Our study presents a detailed parcellation of cortical and subcortical changes. This is important, as cholinergic changes in distinct anatomical regions may contribute to specific clinical symptoms and signs.[53] For example, we observed severe loss of cholinergic terminals in key structures of central auditory pathways including the inferior colliculus, medial geniculate nucleus, and primary auditory cortex (Heschl's gyrus). This is interesting, as previous studies reported perturbation in auditory attention and central auditory processing in Dementia with Lewy bodies.[54,55] Collectively, this may suggest a substantial cholinergic component in dysfunctional auditory processing in Dementia with Lewy bodies. Another example is the posterior ventrolateral nucleus (VLp) of the thalamus, which showed a large group difference measured in effect size (Table 3). The VLp is involved in motor function and tremor, and is investigated as a target for deep brain stimulation in Lewy body disease.[56] Our findings support that the VLp is affected in Lewy body disease, and that there may be a cholinergic component to its dysfunction.

Neuropathological studies have suggested that the dorsal motor nucleus of the vagus nerve is involved early in many cases of Lewy body disease.[57,58] Interestingly, we found lower [^{18}F]FEOBV uptake in a brainstem region overlapping this nucleus (VSM) (Table 3). In isolated REM sleep behavior disorder, a prodromal stage of Dementia with Lewy bodies and Parkinson's disease, there is pathology in the subcoerulean nucleus, which is localized very close to the cholinergic projection neurons in the pedunculopontine nucleus.[59,60] Here, we demonstrate specific cholinergic changes in regions corresponding to both brainstem nuclei, and in distinct thalamic nuclei receiving cholinergic afferents from the pedunculopontine nucleus. As such, detailed cholinergic anatomical mapping using [^{18}F]FEOBV PET could be a valuable tool for investigating progression patterns in Lewy body disease.

In Dementia with Lewy bodies patients, the somatomotor and salience networks were the most affected cortical networks (Table 3). The somatomotor network is implicated in somatosensory and somatomotor functions, and the salience network is important for attentional function. Accordingly, the Dementia with Lewy bodies patients had impaired motor function, and attention was the most affected cognitive domain. Interestingly, the somatomotor and salience networks have high functional connectivity with the posterior basal forebrain,[30] which showed a decreased volume in our Dementia with Lewy bodies patients. By contrast, the limbic network was the least affected cortical network (Table 3). The limbic network is implicated in memory function, which was among the least affected cognitive domains in our Dementia with Lewy bodies patients (Table 2). Correspondingly, the limbic network has high functional connectivity with the anterior basal forebrain, which showed less atrophy compared to the posterior basal forebrain in Dementia with Lewy bodies patients (Fig. 3B). As such, loss of cholinergic integrity in cortical networks seems to align with both symptomatology and subregional atrophy of the basal forebrain.

In Dementia with Lewy bodies patients, we found relevant voxel-wise correlations between [^{18}F]FEOBV uptake and memory and attentional function. Surprisingly, no correlations were found between cortical [^{18}F]FEOBV uptake and language or visuospatial function, or between measures of global cognitive function and cortical [^{18}F]FEOBV uptake. Compared to healthy control subjects, the [^{18}F]FEOBV uptake in Dementia with Lewy bodies patients generally showed lower variance and skewed distribution (Fig. 2A, top row). This could imply that the cholinergic terminals in these regions are nearly depleted. Therefore, it may not be ideal to study associations between cortical cholinergic system changes and cognitive function in Lewy body patients with dementia.[61]

Distinct cholinergic differences between Dementia with Lewy bodies and Parkinson's disease patients

We found that the cholinergic terminal loss in newly diagnosed Dementia with Lewy bodies

patients was considerably more widespread compared to a group of Parkinson's disease patients 8 years after their diagnosis. There were no regions with lower uptake in Parkinson's disease patients compared to Dementia with Lewy bodies patients, and the affected regions in Parkinson's disease patients were equally or more affected in Dementia with Lewy bodies patients. Thus, Dementia with Lewy bodies seems to be a subtype of Lewy body disease characterized by severe cholinergic degeneration manifesting in parallel to dopaminergic degeneration.

In Dementia with Lewy bodies compared to Parkinson's disease patients, the smallest differences in [^{18}F]FEOBV uptake were found in occipital regions. This implies that cholinergic terminals in posterior cortical regions are affected early in Lewy body disease, and that these changes are not sufficient to cause dementia. The largest group differences were found in more anteriorly located regions, particularly limbic regions. This suggests that loss of cholinergic integrity in limbic regions could contribute to the development of dementia. Accordingly, a recent serial PET study assessing longitudinal changes in cortical acetylcholinesterase activity in Parkinson's disease patients found that cortical changes were most severe in posterior regions at baseline and progressed to involve more anteriorly located regions over time.[62] This suggests that the cholinergic changes in Parkinson's disease evolve over time and become increasingly similar to those in Parkinson's disease dementia and Dementia with Lewy bodies.

Our results extend previous PET and SPECT studies on cholinergic changes in Lewy body diseases, which mainly used substrate tracers for acetylcholinesterase (78% of all studies; see Supplementary Table 4). Together these studies found cholinergic changes across isolated REM sleep behavior disorder, Parkinson's disease, Parkinson's disease dementia, and Dementia with Lewy bodies. The cholinergic changes were most pronounced in Dementia with Lewy bodies and Parkinson's disease dementia, and least in isolated REM sleep behavior disorder (Fig. 5). Across all studies, there was a strong correlation between cholinergic loss and decreasing cognitive function (r_s=0.7611, P<0.0001).

Atrophy of the basal forebrain and cholinergic terminal loss in Lewy body disease

This study measured concomitant proxy markers of degeneration of cholinergic nuclei in the basal forebrain and terminal deficits in cholinergic projecting neurons. The Dementia with Lewy bodies group showed reduced cortical [^{18}F]FEOBV PET uptake corresponding with lower posterior basal forebrain volumes. This finding corroborates a post-mortem study of Dementia with Lewy bodies patients that found a correlation between cell loss in the posterior basal forebrain and cortical levels of choline acetyltransferase, a protein specific to cholinergic neurons.[1,63] Notably, the correlations between the posterior basal forebrain volume and [^{18}F]FEOBV uptake in Dementia with Lewy bodies patients showed a similar spatial distribution compared to the voxel-wise comparison of [^{18}F]FEOBV uptake in Dementia with Lewy bodies patients and healthy control subjects (Fig. 3D versus Fig. 2B). This is interesting because it supports the hypothesis that degeneration of cholinergic projecting neurons bears the brunt of cholinergic terminal loss in Dementia with Lewy bodies.

In our study, the anterior basal forebrain volume was decreased in Dementia with Lewy bodies, but not in Parkinson's disease. The anterior basal forebrain provides cholinergic innervation to the hippocampus, hypothalamus, olfactory cortex, and limbic structures.[64] Accordingly, the differences in [^{18}F]FEOBV uptake between the Dementia with Lewy bodies and Parkinson's disease groups were most pronounced in the hippocampus and limbic structures. This indicates a good correspondence between proxy measures of cell body degeneration and terminal loss in cholinergic projecting neurons in patients with Lewy body disease. Also, it suggests that pathological processes in the anterior basal forebrain and corresponding limbic cholinergic dysfunction is important for development of dementia.

In line with our results, correlations between basal forebrain atrophy and cortical cholinergic denervation measured with [^{18}F]FEOBV PET have also been reported in patients with Alzheimer's disease.[65]

We previously reported decreased cortical [^{18}F]FEOBV PET uptake in the exact same sample of Parkinson's disease patients.[8] Interestingly, we here demonstrate that these Parkinson's disease

patients have preserved posterior basal forebrain volumes. This apparent discrepancy supports that cholinergic terminal loss precedes cell death. The finding aligns with a post-mortem study in Parkinson's disease patients reporting non-significant reductions in the posterior basal forebrain and reduced cortical choline acetyltransferase activity.[66]

In contrast with our findings, a recent study on a large sample of Parkinson's disease patients found that subregional atrophy of the basal forebrain correlates with [^{18}F]FEOBV uptake in several cortical regions, although not in posteriorly located regions.[67] Our findings suggest that this may explained by floor effects in posterior regions (Fig. 2A). Although not significant in our smaller sample, we found a similar correlation coefficient for posterior basal forebrain volumes and cortical [^{18}F]FEOBV uptake.

Together, these findings support that cholinergic terminal degeneration precedes overt neuronal cell loss, that basal forebrain pathology is associated with cognitive decline in Lewy body disease, and that a proportion of Parkinson's disease patients are at risk of cognitive decline despite having preserved basal forebrain volumes.[68,69] The phenomenon that terminal dysfunction and degeneration precedes cell loss appears to be universal in Lewy body disease, as it has also been observed in the nigrostriatal dopamine system, the locus coeruleus, and the sympathetic innervation of the heart.[70-72]

Asymmetrical cholinergic degeneration and its relationship with motor performance and brain metabolism

There was a good correlation between the interhemispheric asymmetry index of [^{18}F]FEOBV PET uptake and the asymmetry index of motor performance on the Alternate Tapping Test (Fig. 4A). This translates to a better motor performance in the extremity contralateral to the cerebral hemisphere with more preserved cholinergic terminals. This finding may have two possible interpretations. Either it could point to an important cholinergic component of motor function.[73] Or the correlation may be confounded by degeneration of other neurotransmitter systems, such as the dopaminergic. If so, it would imply that degeneration and dysfunction of the cholinergic system is linked anatomically with these other neurotransmitter systems.[6] Regardless of the

mechanism, our findings show that the cholinergic system is more affected in the ipsilateral compared to the contralateral hemisphere. As neurons in the cerebral hemispheres have approximately 100-fold more ipsilateral than contralateral connections, this finding could support that the pathological process spreads via neuronal synapses.[16] This would also predict that the ipsilateral dopaminergic system would be more affected, and hence provide a good explanation for the correlation between motor function and [^{18}F]FEOBV PET uptake. Lastly, we found a good correlation between interhemispheric [^{18}F]FEOBV and [^{18}F]FDG PET uptake (Fig. 4B), suggesting that the loss of cholinergic input contributes to hypometabolism.[12]

Several patients were unable to participate in the study due to the demands of the two-day testing process, most often because of physical or mental limitations, transportation challenges, and lack of support. This could lead to a bias in the patient sample, favoring better cognitive function, more caregiver support, and better overall health. In addition, the inclusion of biomarkers in the eligibility criteria could result in an intentional bias towards less Alzheimer copathology.[74] Collectively, these biases may have reduced the group differences. However, despite these limitations, our patients with Dementia with Lewy bodies had similar age, sex, education, and cognitive profile as a large, autopsy-confirmed cohort.[75] Another limitation was the use of the RBDSQ questionnaire to assess probable REM sleep behavior disorder instead of video-polysomnography.

In conclusion, this study provides robust evidence for severe cholinergic terminal loss in a newly diagnosed and well-characterized cohort of Dementia with Lewy bodies patients using [^{18}F]FEOBV PET. The cholinergic changes are extensive and regionally distinct, and some brain regions showed very good discriminative potential. The cholinergic changes were quantified in small subcortical structures that may be relevant for understanding disease progression and the cholinergic contribution to symptoms and signs. The study presents the hitherto largest sample of Dementia with Lewy bodies patients in *in vivo* cholinergic molecular research. As such, our findings corroborate [^{18}F]FEOBV PET as a valuable tool in Lewy body research, and even suggest that [^{18}F]FEOBV PET has potential as a diagnostic biomarker. Our results also support that cholinergic terminal loss occurs well before neuronal degeneration of the cortically-

projecting basal forebrain neurons. Therefore, [^{18}F]FEOBV PET may be particularly interesting for studies in prodromal Lewy body disease. Finally, our findings imply that degeneration of the cholinergic system leads to perturbed brain metabolism and may be linked to degeneration of other transmitter systems, such as the dopaminergic. This has implications for understanding asymmetry and disease progression in Lewy body disease.

Funding

Aase og Ejnar Danielsens fond (36456) (JH). "Miguel Servet" program (CP19/00031) (MG). Instituto de Salud Carlos III-Fondo Europeo de Desarrollo Regional (PI20/00613) (MG). Lundbeck foundation (R-359-2020-2533) (PB). Michael J Fox Foundation (MJFF-022856) (PB).

Competing interests

The authors report no competing interests.

Supplementary material

Supplementary material is available at *Brain* online.

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Figure legends

Figure 1 Averaged axial brain slices of healthy control subjects compared to Dementia with Lewy bodies patients and Parkinson's disease patients imaged with [^{18}F]FEOBV PET. The [^{18}F]FEOBV PET images are displayed for 15 healthy control (HC) subjects (top row), 15 Parkinson's disease (PD) patients (middle row) and 25 Dementia with Lewy bodies (DLB) patients (bottom row). Abbreviations: AC, anterior cingulate gyrus; AM, amygdala; CAU, caudate; ENT, entorhinal cortex; HES, Heschl's gyrus; HIP, hippocampus; LGN, lateral geniculate nucleus; OLF, olfactory cortex; PC, posterior cingulate; POS, postcentral gyrus; PRE, precentral gyrus; PUT, putamen; THA, thalamus; and VER, cerebellar vermis. Red color indicates SBR above 3.

Figure 2 Cholinergic terminal loss in Dementia with Lewy bodies patients compared to healthy control subjects and Parkinson's disease patients. The average [^{18}F]FEOBV PET uptake was compared in 25 Dementia with Lewy bodies (DLB) patients, 15 healthy control (HC) subjects, and 15 Parkinson's disease (PD) patients without dementia. **(A)** Examples of regions characterized by high effect size in DLB compared to HC (first row), regions with high effect size in DLB compared to PD (middle row), and regions with low effect size in DLB compared to PD (bottom row). For DLB data in scatterplots, triangles mark possible DLB patients and circles mark probable DLB patients. **(B)** Voxel-wise comparison of [^{18}F]FEOBV PET uptake in DLB and HC (top), and a voxel-wise comparison between DLB and PD (bottom), both thresholded at $P<0.05$, FDR-corrected. The pink arrows mark the color of T-values at the statistical threshold: $T=1.74$ (top) and $T=1.95$ (bottom). Abbreviations: SM, somatomotor network.

Figure 3 Cholinergic basal forebrain volumes in Lewy body disease and correlations with cholinergic terminal loss. (A) MNI-atlas of the anterior (yellow) and posterior (light blue) basal forebrain superimposed on the MNI-template. **(B)** Volumes of the anterior and posterior cholinergic basal forebrain (cBF) in 15 healthy control (HC) subjects, 15 Parkinson's disease (PD) patients, and 25 Dementia with Lewy bodies (DLB) patients with medians and interquartile range. **(C)** Correlations between anterior basal forebrain volumes and olfactory cortex

[^{18}F]FEOBV PET SBR (top), and between posterior basal forebrain volumes and global cortical [^{18}F]FEOBV PET SBR (bottom). **(D)** Parametric T-maps from a voxel-wise regression analysis of [^{18}F]FEOBV PET uptake with the anterior (top) and posterior (bottom) basal forebrain volumes as regressors. The arrows mark the color of T-values at a statistical threshold of $P<0.05$, FDR-corrected, for the anterior (yellow arrow, $T=2.19$) and posterior (light blue arrow, $T=1.97$) basal forebrain. For DLB data in scatterplots, triangles mark possible DLB patients and circles mark probable DLB patients. *$P<0.05$. **$P<0.005$. ***$P<0.001$.

Figure 4 Asymmetry in motor function, cholinergic terminals, and brain metabolism. (A) Correlation between asymmetry in motor function and [^{18}F]FEOBV PET uptake in Dementia with Lewy bodies patients. Subjects in the lower right quadrant have better performance with their left hand and higher [^{18}F]FEOBV PET uptake in the right hemisphere, and conversely for data points in the upper left quadrant. **(B)** Correlation between [^{18}F]FEOBV PET and [^{18}F]FDG PET. Subjects in the upper right quadrant have higher [^{18}F]FEOBV and [^{18}F]FDG PET uptake in the right hemisphere, and conversely for data points in the lower left quadrant. *$P<0.05$. **$P<0.005$. ***$P<0.001$.

Figure 5 Relative loss of *in vivo* cholinergic cortical integrity in Lewy body disease. The figure presents the relative cortical loss in isolated REM sleep behavior disorder (iRBD), Parkinson's disease (PD), Parkinson's disease dementia (PDD), and Dementia with Lewy bodies (DLB) patients compared to healthy control (HC) subjects reported in previous cholinergic PET and SPECT studies. The included studies used pre-synaptic cholinergic tracers for the vesicular acetylcholine transporter and acetylcholinesterase. The studies report global cortical uptake values relative to a control group, and MMSE or MoCA for the patient group. The studies are organized according to diagnosis and sorted within each diagnostic category based on cognitive function. References cited from *left* to *right*: Gersel-Stokholm *et al.*,[6] Bohnen *et al.*,[76] Bohnen *et al.*,[77] Klein *et al.*,[12] Liu *et al.*,[78] Bohnen *et al.*,[79] Kuhl *et al.*,[3] Hilker *et al.*,[80] Shimada *et al.*,[5] Horsager *et al.*,[8] Bohnen *et al.*,[4] Muller *et al.*,[81] Shinotoh *et al.*,[82] Bohnen *et al.*,[4] Bohnen *et al.*,[79] Hiraoka *et al.*,[83] Bohnen *et al.*,[4] Hilker *et al.*,[80] Klein *et al.*,[12] Kuhl *et al.*,[3] Klein *et al.*,[12] Shimada *et al.*,[5] Shimada *et al.*,[5] Nejad-Davarani *et al.*,[10] Shimada *et al.*[84]

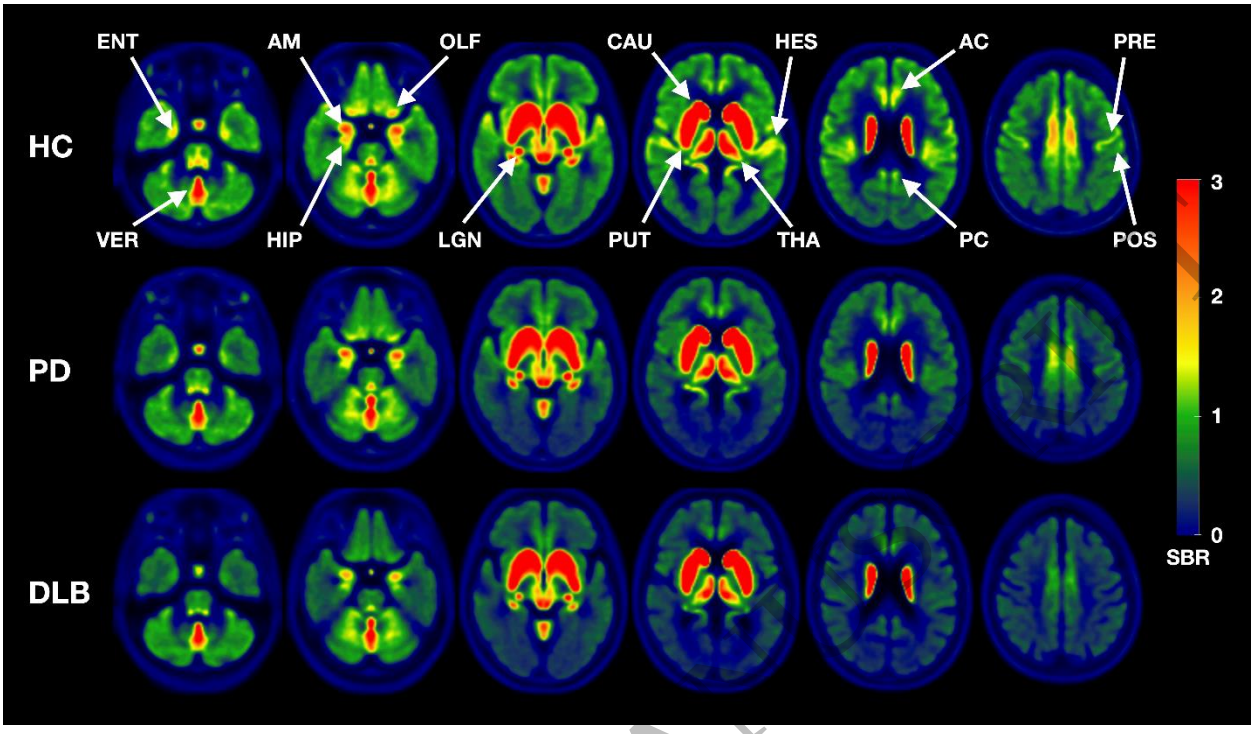

Figure 1
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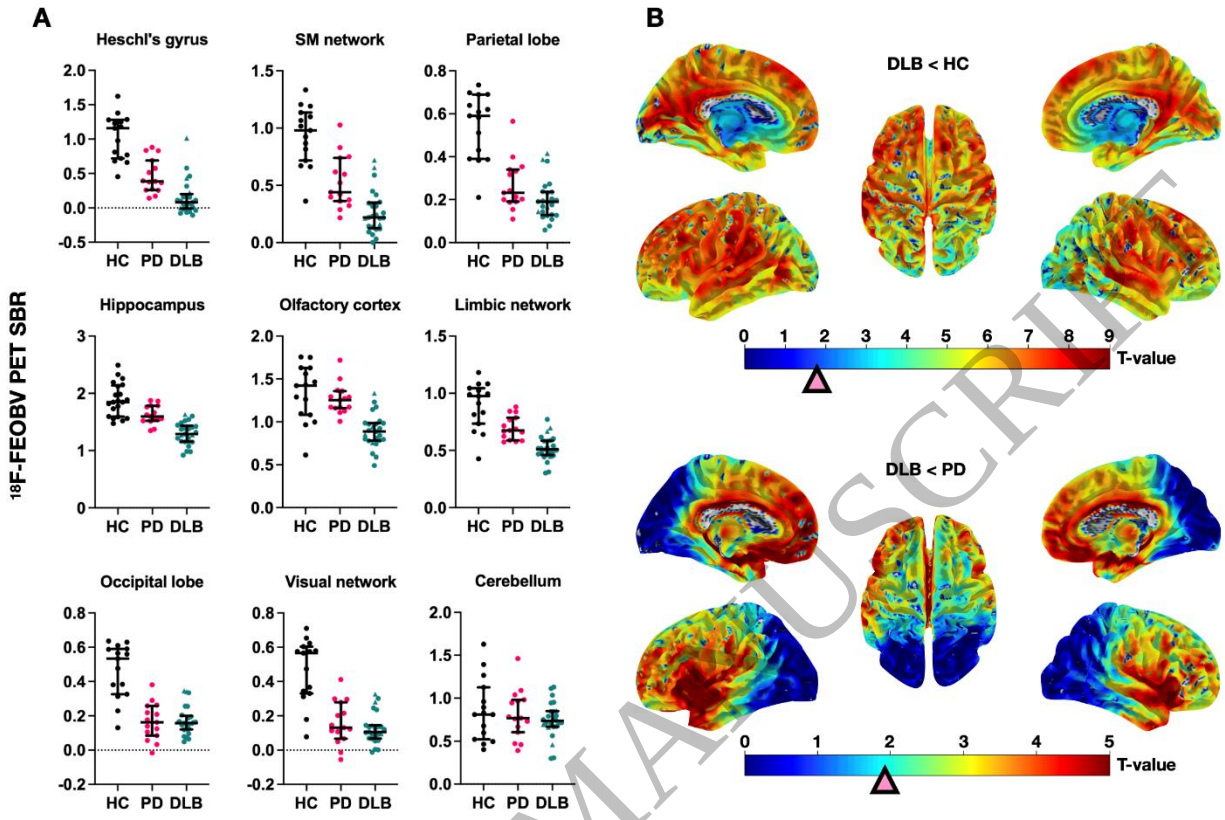

Figure 2
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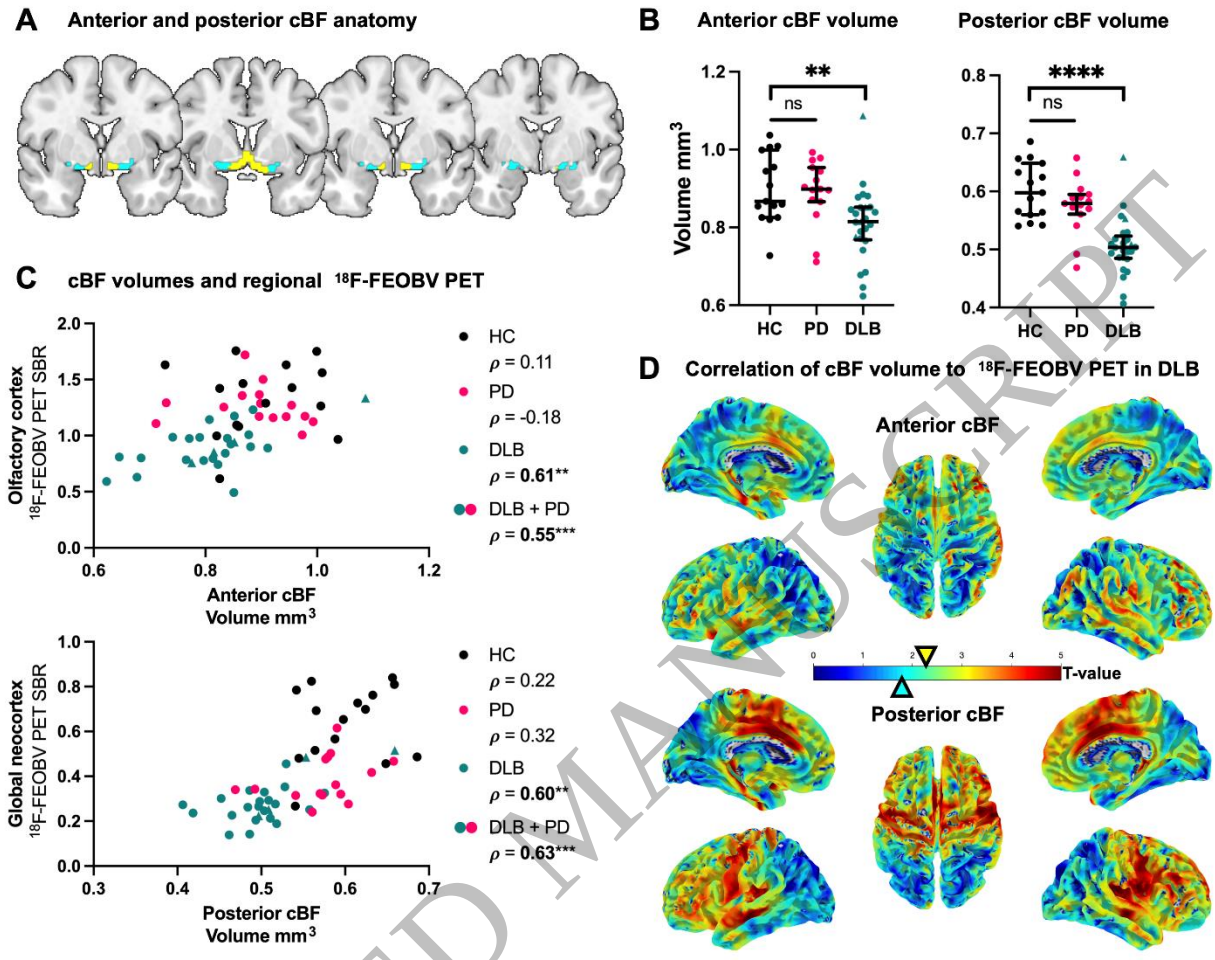

Figure 3
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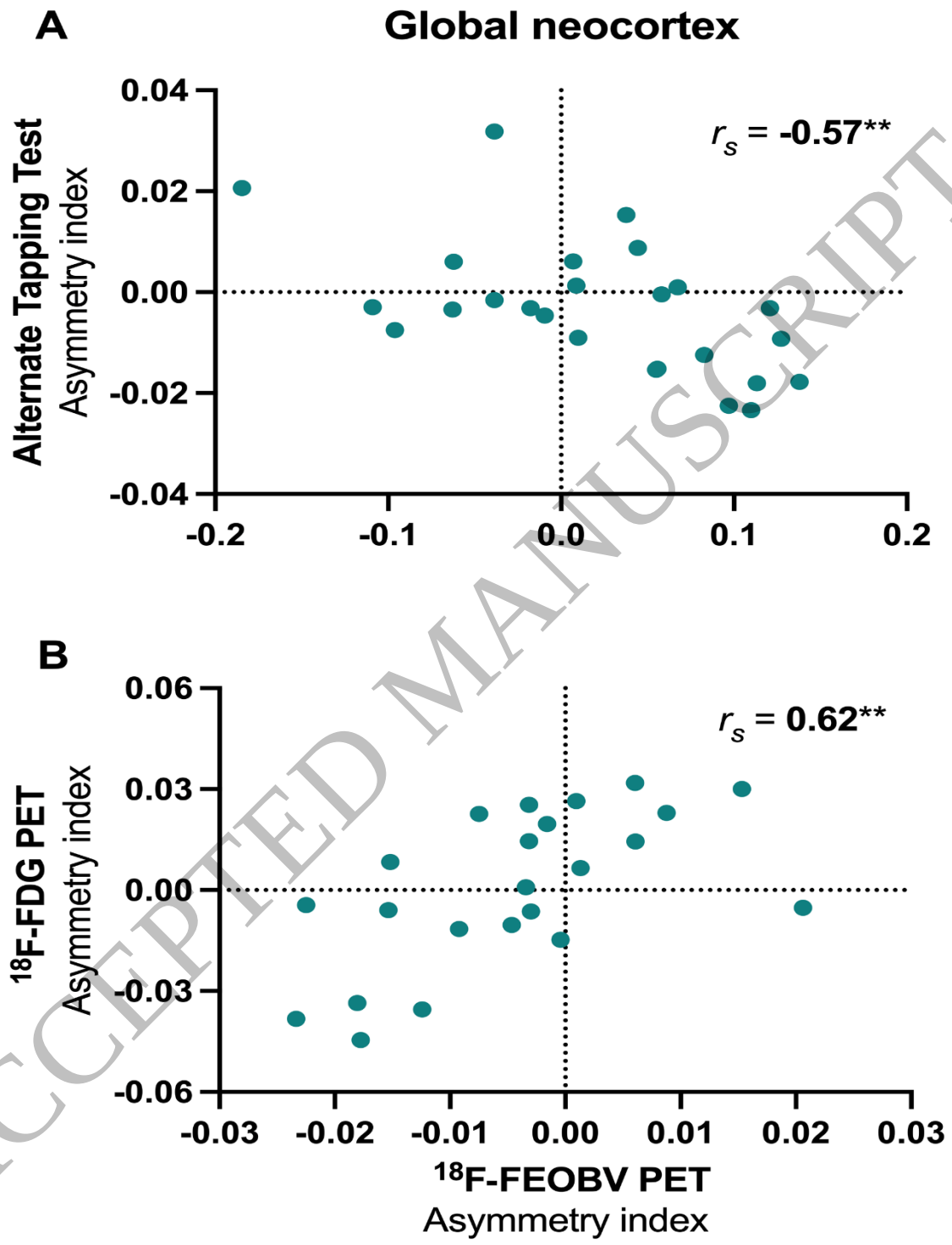

Figure 4
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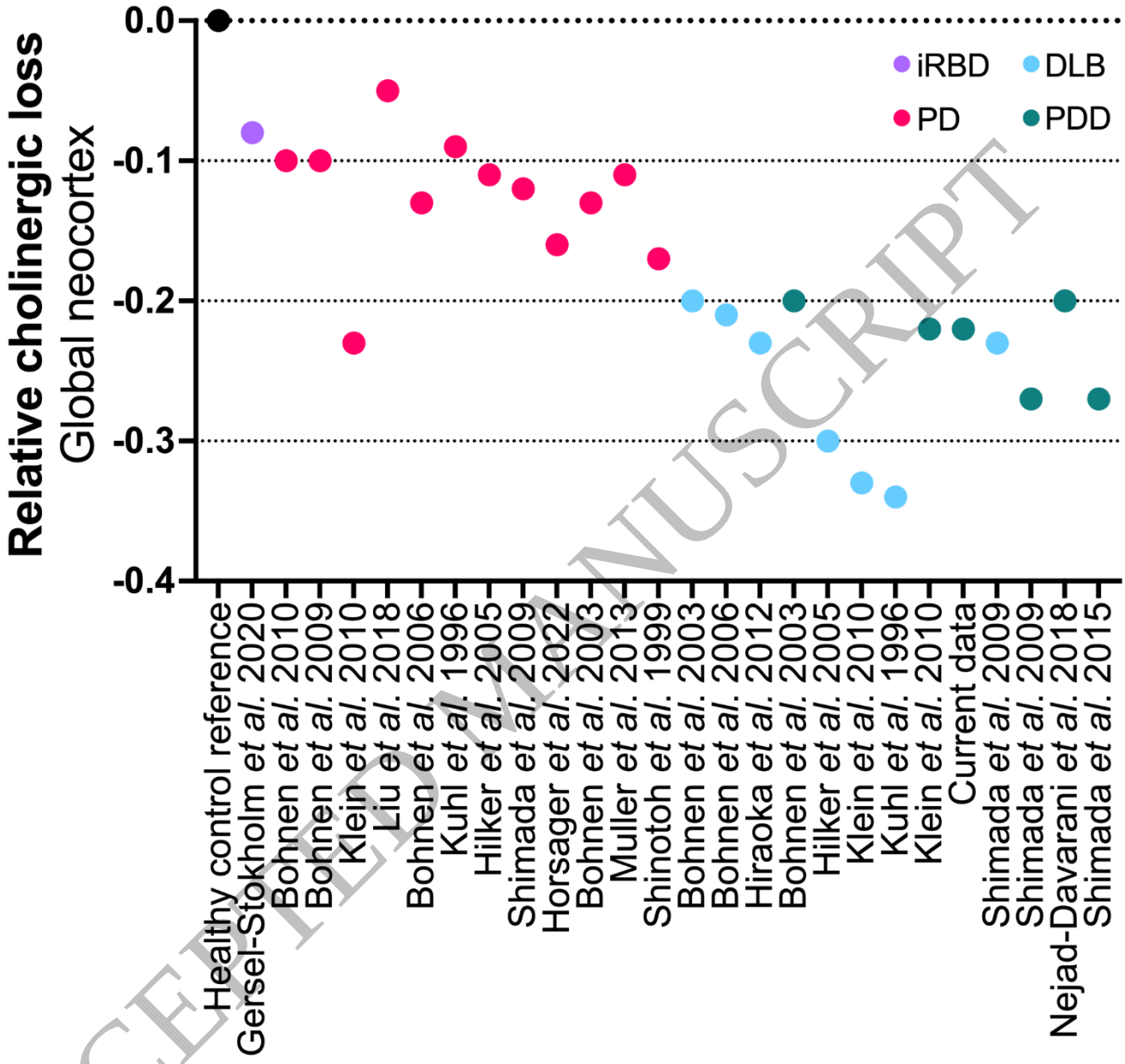

Figure 5
177x166 mm (x DPI)

Table 1 Demographic and clinical information

	HC (*n* = 15)	DLB (*n* = 25)	PD (*n* = 15)	DLB vs HC[a]	DLB vs PD[a]
Age, years	75 ± 6	74 ± 5	70 ± 7	0.6899	0.0719
Sex, male	10 (67%)	21 (84%)	9 (60%)	0.2555	0.1346
Time since diagnosis, years	-	0.36 (0.92)	8.0 (4.0)	-	**<0.0001**
Ethnicity, Caucasian	15 (100%)	25 (100%)	15 (100%)	-	-
Smoking, previous or current	7 (47%)	18 (72%)	6 (40%)	0.1091	0.0939
Smoking, pack years	15 (25)	15 (30)	10 (18)	0.6161	0.4634

Alcohol, units/week	6 (12)	2 (3)	3 (3)	**0.0202**	0.4044
Education, years	15 (8)	14 (3)	–	0.1597	–
Parkinson medication	–	8 (32%)[b]	15 (100%)	–	–
Time on Parkinson mediation, years	–	0.69 (0.51)	–	–	–
LEDD, mg	–	356 ± 135	955 ± 383	–	–
Dementia medication	–	21 (84%)[c]	–	–	–
Time on dementia medication, years	–	0.29 (0.89)	–	–	–
Core clinical features (I/II/III/IV)	–	4/6/8/7	–	–	–
Probable DLB	–	21 (84%)	–	–	–
Possible DLB	–	4 (16%)	–	–	–
Motor assessment					
Dominant hand, right	14 (93%)	25 (100%)	14 (93%)	0.1910	0.1910
UPDRS-III, score	14 ± 6	42 ± 20[d]	32 ± 10[e]	**<0.0001**	0.0706
Parkinsonism	–	19 (76%)	15 (100%)	–	–
H&Y 0/I/II/III	–	6/0/17/2	0/0/11/3/1	–	–
ATT, dominant hand	143 ± 18	106 ± 25	–	**<0.0001**	**–**
ATT, non-dominant hand	134 ± 14	104 ± 23	–	**<0.0001**	**–**
Non-motor assessment					
Ortostatic hypotension	4 (27%)	16 (64%)	6 (40%)	**0.0222**	0.1942
Max systolic drop, mmHg	−8 (17)	−30 (28)	−16 (36)	**0.0025**	**0.0236**
Max diastolic drop, mmHg	+1 (12)	−9 (13)	+3 (12)	**0.0002**	**0.0007**
Max pulse increase, BPM	16 (8)	12 (7)[f]	14 (10)[g]	**0.0019**	0.4327
Odor identification, score	11 (3)	5 (3)	6 (5)	**<0.0001**	**0.0361**
RBDSQ, score	3 (2)	11 (5)	5 (5)	**<0.0001**	**0.0035**
RBD, possible	2 (13%)	22 (88%)	7 (47%)	**<0.0001**	**0.0090**
MFQ, score	0 (1)	3 (1)	–	**<0.0001**	–
Fluctuations	1 (7%)	14 (56%)	–	**0.0018**	–
Hallucinations	0 (0%)	13 (52%)	–	**0.0007**	–
FM-100, total error score	52 (52)	192 (144)[h]	–	**<0.0001**	–
GDS-15, score	0 (2)	4 (3)	–	**<0.0001**	–
Depression	0 (0%)	9 (36%)	–	**0.0083**	–
NMSS, score	11 (21)	72 (59)	53 (47)	**<0.0001**	0.2466
SCOPA-AUT, score	8 (8)	18 (10)	16 (9)	**<0.0001**	0.2812

Presentation of basic demographic and clinical variables in 15 healthy control (HC) subjects, 25 Dementia with Lewy bodies (DLB) patients, and 15 Parkinson's disease (PD) patients with statistical group comparisons. Continuous variables are reported as mean ± *SD* if normally distributed and median (*IQR*) if non-normally distributed. Categorical variables are presented with frequency and percentage. Abbreviations: ATT, Alternate Tapping Test; H&Y, Hoehn and Yahr scale; BPM, beats per minute; LEDD, levodopa equivalent daily dose; NMSS, Non-Motor Symptoms Scale for Parkinson's Disease; SCOPA-AUT, Scales for Outcomes in Parkinson's Disease – Autonomic Dysfunction.

[a]P-values are calculated with unpaired t-test or Mann-Whitney test for continuous variables and Fisher's exact test for categorical variables. Uncorrected P-values < 0.05 are marked in bold.

[b]All DLB patients were treated exclusively with levodopa.

[c]All DLB patients were treated exclusively with cholinesterase inhibitors.

[d]DLB patients were tested while *on* dopaminergic medication.

[e]PD patients were tested while *off* dopaminergic medication.

[f]Two DLB patients were excluded due to irregular pulse.

[g]One PD patient was excluded due to irregular pulse.

[h]One DLB did not complete this task, and one DLB was excluded due to congenital red-green color blindness.

Table 2 Cognitive assessment in DLB patients and HC subjects

Domain	Cognitive test	HC	DLB	*P*
Global	MoCA	28 (2)	19 (7)	**<0.0001**
Z-score		–	−6.1 (4.4)	–
Premorbid	Danish Adult Reading Test	36 (11)	27 (9)	**0.0011**
Z-score		–	−1.0 (1.0)	–
Memory	Hopkins Verbal Learning Test – Revised			
	Total recall	26 (6)	16 (8)	**<0.0001**
	Delayed recall	7 (3)	0 (4)	**<0.0001**
	Recognition	11 (2)	10 (4)	**0.0308**
	Location Learning Test			
	Total displacement[a]	9 (11)	31 (46)	**0.0004**
	Delayed displacement[b]	0 (1)	4 (9)	**0.0002**
Z-score[c]		–	−2.4 (1.9)	-
Attention	Letter-Number Sequencing	11 (3)	6 (5)[d]	**<0.0001**
Z-score		–	−4.0 (1.7)	-
Executive	Phonemic Verbal Fluency[e]	18 (3)	10 (8)	**<0.0001**
	Alternating Verbal Fluency[e]	10 (4)	4 (3)	**<0.0001**
	Wisconsin Cart Sorting Test[f,g]	6 (5)	21 (27)	**<0.0001**
Z-score[c]		–	−3.4 (3.5)	-
Language	Boston Naming Test 30-item	28 (2)	25 (6)	**0.0083**
	Semantic Verbal Fluency[e]	27 ± 6	15 ± 6	**<0.0001**
Z-score[c]		–	−1.6 (2.0)	-
Visuospatial	Royal Clox	11 (1)	10 (3)	**<0.0001**
	Line Orientation[g]	26 (5)	19 (10)	**<0.0001**
	Visual Object and Space Perception			
	Incomplete letters	20 (1)	18 (2)	**0.0005**
	Silhouettes	17 ± 1	14 ± 2	**<0.0001**
	Cube Analysis	10 (0)	9 (2)	**<0.0001**
	Pareidolia	1 (1)	4 (6)	**0.0002**
Z-score[c]		–	−3.0 (3.9)	-
Total Z-score		-	−3.4 (2.2)	–

Cognitive assessment in 15 healthy control (HC) subjects and 24 Dementia with Lewy bodies (DLB) patients. The test results of one DLB patient were not included in the analysis because the patient acknowledged having prior knowledge of and practicing multiple tests before the actual examination. Continuous variables are reported as mean ± *SD* if normally distributed and median (*IQR*) if non-normally distributed. Categorical variables are presented with frequency and percentage. P-values (p) are calculated with unpaired t-test or Mann-Whitney test for continuous variables.

[a]Sum of displacement scores for each picture from its correct location on trials 1-5.

[b]Displacement score for each picture from its correct location on delayed recall trial.

[c]Composite Z-score.

[d]One DLB patient did not complete his test.

[e]Total correct.

[f]Total errors.

[g]Two DLB patients did not complete this test.

Table 3. Regional specific binding ratios of [^{18}F]FEOBV PET in DLB patients and comparisons with PD patients and HC subjects

Region	HC	DLB	PD	DLB < HC			DLB < PD		
	SBR ± *SD*	**SBR ± *SD***	**SBR ± *SD***	***RD***	***d***	***P***	***RD***	***d***	***P***
Striatum									
Caudate	7.09 ± 1.10	5.67 ± 0.97	6.42 ± 1.01	−20	−1.36	**0.0001**	−12	−0.76	0.0244
Putamen	9.60 ± 1.34	8.26 ± 1.03	8.69 ± 1.24	−14	−1.12	**0.0011**	−4.0	−0.38	0.2418
Accumbens	6.15 ± 0.78	5.71 ± 0.83	5.90 ± 0.78	−7.2	−0.55	0.1009	−3.3	−0.24	0.4641

Thalamus									
VLp	1.85 ± 0.41	1.27 ± 0.31	1.39 ± 0.28	−31	−1.61	**<0.0001**	−8.3	−0.39	0.2496
LGN	4.65 ± 1.10	3.07 ± 0.88	3.94 ± 0.83	−34	−1.58	**<0.0001**	−22	−1.01	0.0039
CM	5.30 ± 0.83	4.22 ± 0.71	4.68 ± 0.68	−20	−1.41	**<0.0001**	−10	−0.66	0.0510
MGN	1.80 ± 0.32	1.39 ± 0.27	1.71 ± 0.19	−23	−1.38	**0.0001**	−18	−1.35	**0.0003**
Hb	0.93 ± 0.52	0.56 ± 0.31	0.97 ± 0.21	−40	−0.86	**0.0077**	−42	−1.54	**<0.0001**
Hippocampus									
	1.86 ± 0.25	1.30 ± 0.19	1.62 ± 0.16	−30	−2.55	**<0.0001**	−20	−1.83	**<0.0001**
Amygdala									
	2.28 ± 0.36	1.76 ± 0.23	2.07 ± 0.14	−23	−1.72	**<0.0001**	−15	−1.61	**<0.0001**
Brainstem									
SubC	1.74 ± 0.35	1.41 ± 0.25	1.49 ± 0.29	−19	−1.08	**0.0013**	−5.6	−0.31	0.3322
SC	2.11 ± 0.48	1.66 ± 0.38	1.92 ± 0.37	−21	−1.03	**0.0024**	−13	−0.69	0.0427
IC	0.55 ± 0.28	0.33 ± 0.20	0.44 ± 0.23	−40	−0.90	**0.0064**	−25	−0.51	0.1181
PTg	1.68 ± 0.23	1.42 ± 0.33	1.51 ± 0.24	−15	−0.89	**0.0132**	−6.0	−0.31	0.3677
LC	1.30 ± 0.32	1.08 ± 0.26	1.37 ± 0.21	−17	−0.76	**0.0215**	−21	−1.21	0.0009
VSM	1.87 ± 0.31	1.66 ± 0.26	1.84 ± 0.26	−12	−0.76	**0.0221**	−10	−0.69	0.0411
Ve	1.36 ± 0.34	1.31 ± 0.30	1.46 ± 0.26	−4.0	−0.17	0.5992	−10	−0.53	0.1187
Cortical lobes									
Parietal	0.54 ± 0.15	0.20 ± 0.09	0.27 ± 0.12	−64	−2.76	**<0.0001**	−48	−1.78	**<0.0001**
Temporal	0.68 ± 0.17	0.32 ± 0.10	0.44 ± 0.10	−53	−2.56	**<0.0001**	−27	−1.18	**0.0008**
Frontal	0.71 ± 0.18	0.33 ± 0.11	0.48 ± 0.10	−53	−2.48	**<0.0001**	−31	−1.41	**0.0001**
Occipital	0.46 ± 0.16	0.17 ± 0.08	0.17 ± 0.11	−63	−2.31	**<0.0001**	3.0	0.05	0.8724
Neocortex	0.64 ± 0.17	0.28 ± 0.09	0.39 ± 0.10	−56	−2.61	**<0.0001**	−27	−1.05	**0.0023**
Cortical networks									
SMN	0.93 ± 0.26	0.26 ± 0.19	0.53 ± 0.23	−72	−2.99	**<0.0001**	−50	−1.26	**0.0003**
Salience	0.93 ± 0.25	0.40 ± 0.15	0.64 ± 0.15	−57	−2.61	**<0.0001**	−37	−1.60	**<0.0001**
Control	0.75 ± 0.21	0.32 ± 0.11	0.47 ± 0.12	−57	−2.56	**<0.0001**	−30	−1.20	**0.0006**
DMN	0.77 ± 0.21	0.35 ± 0.11	0.49 ± 0.12	−54	−2.51	**<0.0001**	−29	−1.23	**0.0005**
DAN	0.61 ± 0.18	0.25 ± 0.10	0.32 ± 0.12	−59	−2.48	**<0.0001**	−21	−0.59	0.0708
VN	0.46 ± 0.19	0.13 ± 0.09	0.16 ± 0.13	−73	−2.32	**<0.0001**	−20	−0.29	0.3564
Limbic	0.89 ± 0.20	0.52 ± 0.11	0.70 ± 0.10	−42	−2.29	**<0.0001**	−26	−1.70	**<0.0001**
Olfactory cortex									
	1.34 ± 0.38	0.89 ± 0.20	1.26 ± 0.18	−33	−1.61	**<0.0001**	−29	−1.99	**<0.0001**
Insula									
	1.51 ± 0.29	0.99 ± 0.17	1.26 ± 0.15	−35	−2.21	**<0.0001**	−18	−1.12	**0.0047**
Heschl's gyrus									
	1.03 ± 0.33	0.15 ± 0.25	0.46 ± 0.25	−85	−3.00	**<0.0001**	−68	−1.26	**0.0004**
Cingulum									
CGp	1.02 ± 0.24	0.49 ± 0.16	0.70 ± 0.17	−52	−2.64	**<0.0001**	−30	−1.30	**0.0002**
CGa	1.08 ± 0.28	0.58 ± 0.18	0.85 ± 0.18	−46	−2.10	**<0.0001**	−31	−1.44	**<0.0001**

Averaged [^{18}F]FEOBV PET SBR values of 25 Dementia with Lewy bodies (DLB) patients and comparisons with 15 healthy control (HC) subjects and 15 Parkinson's disease (PD) patients. SBR values are presented as mean ± standard deviation (*SD*) and compared by relative specific binding difference (*RD*) in percent, Cohen's effect size (*d*) and P-values from students t-tests (*P*). Bold p-values are significant results after FDR-correction. Abbreviations: CGa, anterior cingulate; CGp, posterior cingulate; CM, centromedian nucleus; DAN, dorsal attention network; DMN, default mode network; Hb, habenula; IC, inferior colliculus; LC, locus coeruleus; LDTg_CGPn, laterodorsal tegmental nucleus and central grey of the rhombencephalon; LGN, lateral geniculate nucleus; MGN, medial geniculate nucleus; PTg, pedunculopontine nucleus; SC, superior colliculus; SMN, somatomotor network; SN, substantia nigra; SubC, subcoeruleus; Ve, vestibular nuclei complex; VLp, posterior part of the ventral lateral nucleus; VN, visual network; and VSM, viscero-sensory-motor nuclei complex.