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Impaired cholinergic integrity of the colon and pancreas in dementia with Lewy bodies

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Abstract

Dementia with Lewy bodies is characterized by a high burden of autonomic dysfunction and Lewy pathology in peripheral organs and components of the sympathetic and parasympathetic nervous system. Parasympathetic terminals may be quantified with [^{18}F]fluoroetoxybenzovesamicol, a PET tracer that binds to the vesicular acetylcholine transporter in cholinergic presynaptic terminals. Parasympathetic imaging may be useful for diagnostics, improving our understanding of autonomic dysfunction, and for clarifying the spatiotemporal relationship of neuronal degeneration in prodromal disease. Therefore, we aimed to investigate the cholinergic parasympathetic integrity in peripheral organs and central autonomic regions of subjects with Dementia with Lewy bodies and its association with subjective and objective measures of autonomic dysfunction. We hypothesized that organs with known parasympathetic innervation, especially the pancreas and colon, would have impaired cholinergic integrity.

To achieve these aims, we conducted a cross-sectional comparison study including 23 newly diagnosed non-diabetic subjects with Dementia with Lewy bodies (74 ± 6 years, 83% male) and 21 elderly controls (74 ± 6 years, 67% male). We obtained whole-body images to quantify PET uptake in peripheral organs and brain images to quantify PET uptake in regions of the brainstem and hypothalamus. Autonomic dysfunction was assessed with questionnaires and measurements of orthostatic blood pressure.

Subjects with Dementia with Lewy bodies displayed reduced cholinergic tracer uptake in the pancreas (32% reduction, P = 0.0003) and colon (19% reduction, P = 0.0048), but not in organs with little or no parasympathetic innervation. Tracer uptake in a region of the medulla oblongata overlapping the dorsal motor nucleus of the vagus correlated with autonomic

symptoms (r_s = -0.54, P = 0.0077) and changes in orthostatic blood pressure (r_s = 0.76, P < 0.0001). Tracer uptake in the pedunculopontine region correlated with autonomic symptoms (r_s = -0.52, P = 0.0104) and a measure of non-motor symptoms (r_s = -0.47, P = 0.0230).

In conclusion, our findings provide the first imaging-based evidence of impaired cholinergic integrity of the pancreas and colon in Dementia with Lewy bodies. The observed changes may reflect parasympathetic denervation, implying that this process is initiated well before the point of diagnosis. The findings also support that cholinergic denervation in the brainstem contributes to dysautonomia.

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Abbreviations: [^{18}F]FEOBV = [^{18}F]fluoroetoxybenzovesamicol, NMSS = Non-Motor Symptoms Scale for Parkinson's Disease; SCOPA-AUT = Scales for Outcomes in Parkinson's Disease – Autonomic Dysfunction; UPDRS = Unified Parkinson's Disease Rating Scale

Introduction

Dementia with Lewy bodies is characterized by a high burden of autonomic dysfunction and Lewy pathology in peripheral organs and components of the sympathetic and parasympathetic nervous system.[1-4] Whereas methods for imaging sympathetic denervation are now widely used for research and diagnostic purposes,[5] parasympathetic denervation has never been visualized in living subjects with Dementia with Lewy bodies. Parasympathetic cholinergic terminals can theoretically be measured with [^{18}F]fluoroetoxybenzovesamicol ([^{18}F]FEOBV), a PET tracer that binds to the vesicular acetylcholine transporter in cholinergic presynapses.[6]

Studying the parasympathetic nervous system in Dementia with Lewy bodies is important for at least three reasons. First, quantifying parasympathetic cholinergic denervation in central and peripheral regions of the parasympathetic nervous system may help us understand its contribution to autonomic dysfunction. Second, parasympathetic denervation may differentiate subjects with Dementia with Lewy bodies from those suffering from other neurodegenerative diseases, particularly Alzheimer's disease.[1,7] Third, autonomic dysfunction may develop in prodromal Dementia with Lewy bodies,[3,8] and parasympathetic imaging may therefore be a valuable tool for diagnosing prodromal disease and for understanding the spatiotemporal relationship of neurodegeneration in Lewy body disorders.

For these reasons, we aimed to investigate the cholinergic integrity in peripheral organs and central autonomic regions of subjects with de novo Dementia with Lewy bodies using [^{18}F]FEOBV PET and correlate its uptake with subjective and objective measures of autonomic dysfunction. We hypothesized that [^{18}F]FEOBV uptake would be decreased in peripheral organs with known parasympathetic innervation, i.e. the pancreas and colon.

Materials and methods

Setting and participants

This comparative cross-sectional study was performed at the Department of Nuclear Medicine and PET at Aarhus University Hospital in Denmark between the years 2020 and 2022. We recruited 23 non-diabetic and newly diagnosed subjects with Dementia with Lewy bodies (74 ± 6 years, 83% male) from neurological departments and through advertisement in the Danish Alzheimer's Association members' magazine. Criteria for inclusion comprised a diagnosis of Dementia with Lewy bodies and occipital hypometabolism, with or without the cingulate island sign, on [^{18}F]fluorodeoxyglucose PET and/or reduced striatal dopamine transporter uptake on PET or SPECT, according to current diagnostic criteria.[9] Twenty-one cognitively intact elderly controls (74 ± 6 years, 67% male) were recruited through newspaper advertisement and matched to the cases on age and sex. Criteria for exclusion were diabetes, glycated hemoglobin above 47 mmol/mol and venous fasting glucose above 6.9 mmol/L; severe psychiatric disorder, stroke, peripheral neuropathy, inflammatory bowel disease, celiac disease, organ failure, and alcohol abuse; cancer, surgery, or radiation therapy involving the brain or peripheral organs; and contraindications to MRI. Furthermore, we excluded healthy control subjects with a Montreal Cognitive Assessment (MoCA) score below 26.[10] The sample size was estimated to 24 participants in each group based on group differences in pancreatic [^{18}F]FEOBV uptake presented in a previous study in Parkinson's disease.[11] Reasons for non-participation are presented in Supplementary Fig.1. The study was conducted according to the Declaration of Helsinki and approved by the local ethics committee (project no. 1-10-72-270-19). All participants provided informed signed consent.

Image acquisition

All [^{18}F]FEOBV PET investigations were performed on the same Siemens Biograph Vision 600 PET/CT camera after intravenous injection of approximately 200 MBq [^{18}F]FEOBV. Whole-body images were recorded from 0 to 70 minutes and brain images from 180 to 210 minutes after injection.[6,12] Brain and whole-body CT scans were performed for attenuation correction, including intravenous contrast enhancement for anatomical visualization of internal organs. Participants abstained from food for at least 6 hours and liquids for at least 2 hours before PET. Medications were not paused. T1 MPRAGE MRI brain images were obtained on a 3T Siemens Magnetom Skyra and used for coregistration.

Whole-body image processing

Whole-body [^{18}F]FEOBV PET images were reconstructed with resolution recovery and time-of-flight, four iterations, five subsets, 2 mm Gaussian filtering, 440 matrix, relative scatter correction, attenuation correction, and decay correction to the time of tracer injection. Images obtained 60–70 minutes post injection were averaged and normalized to injected dose and subject weight to calculate standard uptake values (SUV). We previously showed that SUVs in peripheral organs obtained at this time point are strongly correlated with volumes of distribution calculated from kinetic modeling.[11] One additional reason for conducting [^{18}F]FEOBV recordings of the abdominal organs and the brain at separate time points was to prevent any interference from luminal intestinal signals resulting from the excretion of [^{18}F]FEOBV into the bile.[6,11] [^{18}F]FEOBV PET uptake in peripheral organs was calculated in PMOD as previously described.[6] In brief, PET images were fused with a contrast-enhanced whole-body CT scan. Then, standard uptake values in peripheral organs were assessed by manually defining regions with a 8 mm brush size on six adjacent axial slices in the body and tail of the pancreas, left ventricular wall of the heart, kidney cortex, and parenchyma of the spleen and liver.[11] Regions in paraspinal muscle were outlined with 10 mm circles on 20 adjacent axial slices at the lumbar level. The entire colon was outlined on axial slices from the PET and CT images. The final colon CT volume was calculated by subtracting luminal gas defined as Hounsfield units below 300 from the total colon volume. Post-hoc we manually outlined the entire PET signal and normalized it to CT-derived anatomical volumes in the submandibular, parotid, and adrenal glands. All steps involving manual processing were performed blinded to clinical status.

Brain image processing

[^{18}F]FEOBV PET brain images were binned into six frames of five minutes and reconstructed with resolution recovery and time-of-flight, eight iterations, five subsets, 440 matrix, zoom 2, and no filter. The final voxel size was 0.83x0.83x1.65 mm^3 and the spatial resolution was 2 mm full-width half-maximum. PET brain image frames were corrected for head motion and averaged, then co-registered with the T1 images and normalized to the MNI template in SPM12.[13] The PET images were then intensity normalized to the centrum semiovale to yield SUV ratios.[14] We quantified regional tracer uptake by superimposing stereotactic MNI-space atlases to the PET images.[15-18] One of these atlas regions, from now on referred to as the viscero-sensory-motor nuclei complex, encompassed the anatomical location of the solitary nucleus, dorsal motor nucleus of the vagus, hypoglossal nucleus, prepositus, intercalated nucleus, and interpositus.[15]

Clinical assessments

Autonomic symptoms were assessed with the Scales for Outcomes in Parkinson's Disease – Autonomic Dysfunction (SCOPA-AUT) and non-motor symptoms with the Non-Motor Symptoms Scale for Parkinson's Disease (NMSS).[19,20] Both scales measure the frequency and severity of symptoms within the past month. Total scores and subscores were calculated as described in the manuals. Presence or absence of hallucinations was evaluated with the North-Eastern Visual Hallucinations Interview.[21] Fluctuations were defined as a score above 2 on the Mayo Fluctuations Scale, and probable REM Sleep Behavior Disorder as a score above 5 on the REM Sleep Behavior Disorder Screening Questionnaire (RBDSQ).[22,23] Motor function was assessed with the MDS-UPDRS Part III (UPDRS-III).[24] Depressive symptoms were measured with the 15-item Geriatric Depression Scale.[25] We used the Sniffin' Sticks 16-item smell test to measure odor identification, and the Farnsworth-Munsell Color Vision test to quantify color vision.[26,27] Global cognitive function was evaluated with the MoCA. Blood pressure was measured after 15 minutes of supine rest, and then after one, two, and three minutes in the orthostatic position, following the imaging procedures and a meal. Orthostatic hypotension was defined as a fall in systolic pressure of more than 20 mmHg or diastolic pressure of more than 10 mmHg in any of the three measurements in the standing

position.[28] All clinical assessments including venous fasting glucose were performed on the same day as [^{18}F]FEOBV PET. Finally, measurements of glycated hemoglobin were retrieved from medical records. The median time between glycated hemoglobin and [^{18}F]FEOBV PET was 4.0 (2.1 – 7.6) months. Neuropsychological tests for memory, attention, language, visuospatial, and executive function were administered on a separate occasion (Supplementary material).

Analyses and statistics

We compared peripheral organ [^{18}F]FEOBV uptake in subjects with Dementia with Lewy bodies and healthy controls. We also correlated [^{18}F]FEOBV PET uptake in peripheral organs with that in regions of the brainstem and hypothalamus. We restricted the correlational analyses to organs with lower [^{18}F]FEOBV PET uptake. We compared colon volume in the two groups and used linear regression to adjust [^{18}F]FEOBV uptake in the colon for differences in colon volume. We compared organ [^{18}F]FEOBV PET uptake in subjects taking donepezil to those not taking the medication, and the frequency of orthostatic hypotension in subjects taking levodopa to those not taking the medication. We explored correlations between regional [^{18}F]FEOBV PET uptake and measures of autonomic dysfunction. Specifically, we interrogated orthostatic blood pressure changes and scores and subscores on the SCOPA-AUT and NMSS for correlation with [^{18}F]FEOBV PET uptake in peripheral organs, the brainstem and hypothalamus. As a post-hoc analysis, pancreatic [^{18}F]FEOBV uptake was correlated with fasting venous blood glucose levels and glycated hemoglobin. We also compared tracer uptake in the submandibular, parotid, and adrenal glands between healthy controls and subjects with Dementia with Lewy bodies.

The distribution of data was explored with histograms, box plots, and QQ-plots. Group comparisons of continuous variables were conducted with unpaired t-tests for parametric data and Mann-Whitney tests for non-parametric data. Group comparisons of categorical variables were calculated with Fisher's exact test. Continuous variables are reported as mean ± *SD* if normally distributed and median with interquartile range if non-normally distributed. Categorical variables are presented with frequency and percentage. Correlations were calculated using Spearman's rank correlation or Pearson correlation, as appropriate. Statistical significance was defined at $P < 0.05$, and P-values from multiple correlations were analyzed with the two-stage step-up False Discovery Rate approach of Benjamini, Krieger,

and Yekutieli at Q = 1%.[29] Missing clinical or cognitive observations, detailed in the tables, were discarded in the analyses. We used GraphPad Prism 9 and STATA 17 for statistical analyses and presentation of data. PMOD 4.0 and MRIcroGL 13.2.1 were used for visualization of images.

Results

Demographic and clinical information

The groups did not differ on age, sex, smoking habits, or years of education. Also, the groups did not differ on body mass index, venous fasting glucose, glycated hemoglobin, or frequency of prediabetes. The healthy control group had a higher weekly consumption of alcohol (Table 1). Subjects with Dementia with Lewy bodies had more autonomic symptoms and signs, more impaired cognitive function, poorer color vision and odor identification, more symptoms of depression and REM Sleep behavior disorder, and more fluctuations and hallucinations (Table 2). Subjects with Dementia with Lewy bodies had similar levels of supine blood pressure, but more orthostatic hypotension and larger orthostatic decreases in systolic and diastolic blood pressure, as compared to healthy controls. Subjects with orthostatic hypotension did not differ on cardiovascular symptoms measured on the NMSS or SCOPA-AUT compared to subjects without orthostatic hypotension. Among subjects with orthostatic hypotension (n = 16), eight (50%) reported no cardiovascular symptoms on the NMSS and five (31%) reported no cardiovascular symptoms on the SCOPA-AUT. Maximum systolic or diastolic drop did not correlate with cardiovascular symptoms measured on the NMSS or SCOPA-AUT. The frequency of supine hypertension in subjects with orthostatic hypotension (31%) was not different from that in subjects without orthostatic hypotension (29%) (P > 0.99, Fisher's exact test). Subjects with Dementia with Lewy bodies showed impairments in all cognitive domains, most in executive function and least in language function (Supplementary Table 1). Subjects with Dementia with Lewy bodies taking donepezil (n = 19) did not differ from those not taking the medication (n = 4) in terms of age, time since diagnosis, colon volume, or [^{18}F]FEOBV PET uptake in the pancreas, colon, brainstem, or hypothalamus. Also, the proportion of subjects with orthostatic hypotension was not higher among those taking levodopa medication (n = 7) compared to those not taking the medication (n = 16). In a post-hoc analysis, we undertook a re-categorization of the items within the SCOPA-AUT questionnaire with the aim of assessing symptoms associated with

cholinergic deficiency. This involved the calculation of scores for specific symptoms including constipation (items 5 and 6), heat intolerance (item 21), light sensitivity (item 19), and urinary retention (items 10 – 12). After FDR-correction, subjects with Dementia with Lewy bodies demonstrated a higher burden of constipation compared to healthy controls (P < 0.001). No significant group differences were observed in relation to heat intolerance, light sensitivity, or urinary retention (FDR corrected). Notably, the subscores indicative of cholinergic deficiency did not exhibit correlations with symptoms of orthostatic intolerance or changes in orthostatic blood pressure.

[^{18}F]FEOBV PET uptake in peripheral organs

In both groups, [^{18}F]FEOBV PET showed high uptake in the liver; moderate uptake in the pancreas and heart; and low uptake in the renal cortex, spleen, muscle, and colon. Compared to healthy controls, subjects with Dementia with Lewy bodies showed lower [^{18}F]FEOBV PET uptake in the pancreas (32% reduction, P = 0.0003) and colon (19% reduction, P = 0.0048), but not in the spleen, heart, liver, muscle, or kidney (Fig.1 and Table 3). The group difference in pancreatic [^{18}F]FEOBV uptake remained significant after FDR-correction. [^{18}F]FEOBV PET uptake in the pancreas and colon did not correlate with scores or subscores on the SCOPA-AUT or NMSS, maximum systolic or diastolic drop, or regional [^{18}F]FEOBV PET uptake in the brainstem or hypothalamus. [^{18}F]FEOBV PET uptake in the pancreas did not correlate with [^{18}F]FEOBV PET uptake in the colon or with fasting venous blood glucose or glycated hemoglobin. The colon volume was not significantly larger in subjects with Dementia with Lewy bodies compared to healthy controls, 915 (723–1292) cm^3 versus 851 (620–1064) cm^3, P = 0.2709. [^{18}F]FEOBV PET uptake in the colon showed a weak negative correlation with colon volume in healthy control subjects (R^2 = 0.18, P = 0.0565) and subjects with Dementia with Lewy bodies (R^2 = 0.18, P = 0.0417). The group difference in colonic [^{18}F]FEOBV PET uptake remained significant after adjusting for colon volume, and the adjusted group difference was 0.04% smaller compared to the unadjusted estimate. Post-hoc analyses revealed a non-significant decrease in tracer uptake in the submandibular, parotid, and adrenal glands of subjects with Dementia with Lewy bodies (Supplementary Table 2).

Non-motor symptoms and [^{18}F]FEOBV PET uptake in CNS

regions

SCOPA-AUT correlated with [^{18}F]FEOBV PET uptake in the pedunculopontine region (r_s = -0.52, P = 0.0104) and viscero-sensory-motor region (r_s = -0.54, P = 0.0077) (Fig.2A, left). There were no correlations between SCOPA-AUT and [^{18}F]FEOBV PET uptake in the remaining 30 brainstem regions investigated. With FDR-correction there were no correlations between SCOPA-AUT and [^{18}F]FEOBV PET uptake in any brainstem region, or between subscores of the SCOPA-AUT and [^{18}F]FEOBV PET uptake in the viscero-sensory-motor or pedunculopontine region. There were no correlations between SCOPA-AUT and [^{18}F]FEOBV PET uptake in the hypothalamus.

NMSS correlated with [^{18}F]FEOBV PET uptake in the pedunculopontine region (r_s = -0.47, P = 0.0230), but not the viscero-sensory-motor region (r_s = -0.41, P = 0.0505) or any other brainstem region (Fig.2A, right). With FDR-correction there were no significant correlations between NMSS and [^{18}F]FEOBV PET uptake in any brainstem region, or between subscores of the NMSS and [^{18}F]FEOBV PET uptake in the pedunculopontine region. There were no correlations between NMSS and [^{18}F]FEOBV PET uptake in the hypothalamus.

Orthostatic blood pressure and [^{18}F]FEOBV PET uptake in the CNS

Maximum orthostatic systolic drop correlated with [^{18}F]FEOBV PET uptake in the viscero-sensory-motor region, pedunculopontine region, red nucleus region, and regions within the medullary and pontine reticular reformation. After FDR-correction, only the viscero-sensory-motor region remained significant (r_s = 0.76, P < 0.0001) (Fig.3A). Maximum systolic drop did not correlate with [^{18}F]FEOBV PET uptake in the hypothalamus. Maximum diastolic drop correlated with [^{18}F]FEOBV PET uptake in overlapping brainstem regions, most strongly in the viscero-sensory-motor region (r_s = 0.61, P = 0.0020), but none were significant after FDR-correction (Fig.3B). Maximum diastolic drop did not correlate with [^{18}F]FEOBV PET uptake in the hypothalamus. [^{18}F]FEOBV uptake in the viscero-sensory-motor region was lower in subjects with Dementia with Lewy bodies who experienced orthostatic hypotension, in comparison to those without orthostatic hypotension (P = 0.0148, unpaired t-test).

Regional brain uptake values of [^{18}F]FEOBV in Dementia with Lewy bodies were published previously.[30]

Discussion

This study provides first evidence of impaired cholinergic integrity of the pancreas and colon in subjects with Dementia with Lewy bodies. We also found that subjective and objective measures of autonomic dysfunction correlate with loss of cholinergic terminals in brainstem regions involved in autonomic regulation.

Parasympathetic innervation of peripheral organs

We found a highly significant decreased [^{18}F]FEOBV PET uptake in the pancreas (-32%) and colon (-19%). Observing such strong reductions already in de novo subjects implies that parasympathetic denervation is initiated well before the point of diagnosis. The etiology of decreased [^{18}F]FEOBV PET uptake in the pancreas and colon is at present unclear. The pancreas and proximal two-thirds of the colon receive parasympathetic innervation from the dorsal motor nucleus of the vagus, and the distal colon from pre-ganglionic neurons in the sacral part of the intermediolateral cell column.[31-33] Parasympathetic nerves are cholinergic and decreased uptake of the cholinergic PET tracer [^{18}F]FEOBV in these organs may therefore reflect parasympathetic denervation.[6,11] As such, [^{18}F]FEOBV PET may have potential as a biomarker of systemic disease progression in Dementia with Lewy bodies.

Vagal innervation is notably more pronounced in proximal regions of the gastrointestinal tract as opposed to distal regions. Regrettably, due to the significant biliary and gastric secretion of [^{18}F]FEOBV, it was not possible to obtain reliable data from the stomach or small intestine.[11]

To challenge the specificity of our findings, we also investigated [^{18}F]FEOBV PET uptake in organs with no or very little parasympathetic innervation. There is no evidence for parasympathetic innervation of the human spleen, and splenic tracer uptake may reflect binding to red blood cells and immune cells located in the parenchyma.[34,35] A very recent study identified parasympathetic innervation of the renal vasculature and pelvis,[36] but not the

renal cortex, which was the region of interest in this study. In the renal cortex, the tracer may bind to collecting duct cells capable of secreting acetylcholine.[37] There is likely no discernable parasympathetic innervation of the liver,[38] where [^{18}F]FEOBV signal likely pertains to metabolization of tracer in hepatocytes. In skeletal muscle, the vesicular acetylcholine transporter is expressed in somatomotoric and cholinergic sympathetic fibers.[38] In the heart, vagal innervation of the left ventricle is extremely sparse and cardiomyocytes express the vesicular acetylcholine transporter.[39] Therefore, myocardial [^{18}F]FEOBV PET uptake most likely reflects non-neuronal cholinergic cardiomyocytes. Also, subjects with Lewy body dementia have a preserved number of cholinergic neurons in the nucleus ambiguus, the primary source of vagal innervation to the heart.[40] Our finding of no group differences in [^{18}F]FEOBV PET uptake in these organs strengthen the specificity of our findings in the pancreas and colon. Evidently, in peripheral tissues, [^{18}F]FEOBV may bind to several non-neuronal structures, and its uptake needs to be interpreted cautiously. In a post-hoc analysis, we observed a non-significant decrease in [^{18}F]FEOBV uptake in the submandibular and parotid glands of subjects with Dementia with Lewy bodies. This could suggest either relatively preserved parasympathetic innervation of the salivary glands or potential insensitivity of [^{18}F]FEOBV in detecting this innervation.[11] We also noted a non-significant decrease in tracer uptake in the adrenal gland compared to healthy controls. The adrenal glands receive direct innervation from pre-ganglionic cholinergic sympathetic fibers originating in the thoracic spinal cord. This finding may indicate that Dementia with Lewy bodies primarily affects post-ganglionic neurons or that sympathetic cholinergic innervation remains intact in Lewy body disease.[41]

Parasympathetic denervation in Lewy body disease

The present [^{18}F]FEOBV findings in Dementia with Lewy bodies extend our previous [^{11}C]donepezil PET results in subjects with Parkinson's disease, those with isolated REM Sleep Behaviour Disorder, and surgically vagotomized subjects.[42-46] In these studies, significantly reduced colonic [^{11}C]donepezil uptake was a consistent finding across all groups, and reduced pancreatic uptake was seen in subjects with Parkinson's disease at moderate disease stages. That these findings represent parasympathetic denervation is supported by a [^{11}C]donepezil PET validation study in surgically vagotomized subjects, which showed markedly decreased [^{11}C]donepezil uptake in the proximal, vagus-innervated

part of the colon and a (non-significant) reduction in the pancreas.[45]

However, in a recent [^{18}F]FEOBV study of 15 subjects with Parkinson's disease without dementia, who were studied on average 8 years after diagnosis, we found only a 14% reduction in pancreatic uptake and a non-significant 10% reduction in colon uptake.[11] Thus, [^{18}F]FEOBV and [^{11}C]donepezil PET results in abdominal organs are clearly not fully comparable. We speculate that this difference could be caused by differing compensatory regulation of the two target molecules. Briefly, during the early phases of neurodegeneration, the breakdown-enzyme acetylcholinesterase may be down-regulated, and components of the presynaptic cholinergic machinery including the vesicular acetylcholine transporter may be upregulated, as compensatory responses to uphold a functional level of cholinergic neurotransmission. If this is the case, [^{18}F]FEOBV PET would underestimate the true magnitude of neurodegeneration, whereas [^{11}C]donepezil PET would tend to overestimate it. Of note, such up-regulation of [^{18}F]FEOBV signal has been suggested by the finding of higher-than-healthy control levels of [^{18}F]FEOBV in brainstem structures and hippocampus in prodromal and early Parkinson's disease.[47,48] Similar compensatory regulation has also been seen in the nigrostriatal dopamine system of early-to-moderate stage Parkinson's disease.[49]

We have recently proposed a brain-first vs. body-first model of Lewy body disease.[46,50] This model predicts that subjects with Dementia with Lewy bodies predominantly have body-first etiology, since they much more frequently develop REM sleep behaviour disorder, autonomic symptoms, and cardiac denervation on [^{123}I]metaiodobenzylguanidine scans years before diagnosis compared to subjects with Parkinson's disease.[51-57] Thus, we would expect that subjects with Dementia with Lewy bodies exhibit larger reductions of [^{18}F]FEOBV uptake in the colon and pancreas compared to subjects with Parkinson's disease. This would be a plausible explanation for the more significant reductions seen in the present study of Dementia with Lewy bodies compared to our previous findings in Parkinson's disease without dementia, which may have been an enriched brain-first group.[11] A similar difference was seen in our previous [^{11}C]donepezil study, in which subjects with de novo Parkinson's disease of the body-first type showed much larger reductions in colonic signal compared to de novo subjects of the brain-first type.[46]

Thus, with the important caveat that this study employed a cross-sectional design and that

[^{18}F]FEOBV could be a low-specificity marker of parasympathetic degeneration, the present finding of considerably decreased pancreatic and colonic [^{18}F]FEOBV binding in newly diagnosed subjects suggest that parasympathetic denervation may already be evolving during the prodromal phase of Dementia with Lewy bodies. This parasympathetic denervation occurs in parallel with the well-documented severe cardiac sympathetic denervation, which also develops in the prodromal phase in this subtype of Lewy body disorder.

The cholinergic contribution to autonomic dysfunction

We found a relevant negative correlation between subjective autonomic symptoms measured on the SCOPA-AUT and [^{18}F]FEOBV PET uptake in the viscero-sensory-motor region, a medullary brainstem region corresponding to the location of the solitary nucleus, dorsal motor nucleus of the vagus, hypoglossal nucleus, prepositus, intercalated nucleus, and interpositus.[15] However, [^{18}F]FEOBV PET uptake in this region did not correlate with gastrointestinal symptoms measured with the SCOPA-AUT or NMSS. Accordingly, a post-mortem study in subjects with Dementia with Lewy bodies found that loss of cholinergic neurons in the dorsal motor nucleus of the vagus was not associated with gastrointestinal symptoms.[40]

We also found a relevant negative correlation between objective autonomic signs assessed with changes in orthostatic blood pressure and [^{18}F]FEOBV PET uptake in the viscero-sensory-motor region. The apparent correlation between orthostatic blood pressure changes and [^{18}F]FEOBV uptake in the medulla oblongata is not easily explained using the available knowledge of baroreceptor reflexes and projections of the dorsal motor nucleus of the vagus. The solitary nucleus receives parasympathetic viscerosensoric afferent information from baroreceptors in the aorta and carotids.[41] This information is then integrated in autonomic reflex centers in the brainstem and regulates blood pressure through visceromotoric sympathetic and parasympathetic pathways. Therefore, the solitary nucleus is in a key position to regulate baroreceptor reflexes, which could explain why decreased cholinergic integrity in a brain stem region overlapping this region correlates with orthostatic blood pressure changes.[58] However, vagal viscerosensoric afferents do not express alpha-synuclein, and the viscerosensoric solitary nucleus is usually devoid of Lewy pathology, at least in the early stages of Lewy body disease.[59,60]

In subjects with Parkinson's disease, orthostatic hypotension is associated with failure of both the sympathetic and parasympathetic branch of this baroreceptor reflex.[41] The study did not examine sympathetic noradrenergic function, despite the likelihood that orthostatic hypotension is likely to have a neurogenic origin in Lewy body disease. Sympathetic noradrenergic function could have been examined by tracking blood pressure continuously during and after performance of the Valsalva maneuver, measurement of plasma norepinephrine levels during both supine rest and orthostasis, or assessment of the orthostatic change in heart rate relative to a given change in systolic blood pressure.[61] Combining these measurements with [^{18}F]FEOBV PET imaging provides an exciting avenue for future research.

A post-mortem study in subjects with Lewy body dementia found a non-significantly reduced number of catecholaminergic neurons compared to healthy control subjects in the ventrolateral medulla oblongata, a region controlling sympathetic outputs maintaining arterial blood pressure.[62] Also, the number of catecholaminergic neurons did not depend on the presence or absence of orthostatic hypotension. This evidence may suggest that pathology in the pre-ganglionic sympathetic components of the central autonomic system is of lesser importance to understand orthostatic hypotension in Lewy body disorders. As [^{18}F]FEOBV binds primarily to the terminal end of cholinergic axons, and to a lesser extend to cholinergic cell bodies, decreased [^{18}F]FEOBV binding in medulla may reflect loss of cholinergic fibers terminating in this region.[7] In this view, the association between autonomic dysfunction and decreased [^{18}F]FEOBV uptake in the medulla may be explained by changes in cholinergic nuclei innervating this region, such as the pedunculopontine nucleus.[63] Another possibility may be that [^{18}F]FEOBV uptake in the viscero-sensory-motor region can be considered a surrogate marker of more general functional integrity of baroreflex centers in the medulla oblongata.

[^{18}F]FEOBV PET uptake in a brainstem region corresponding to the pedunculopontine nucleus correlated with non-motor symptoms measured on the NMSS. The NMSS assesses a broad range of non-motor symptoms pertaining to cognition, sleep, autonomic dysfunction, perception, and mood.[20] Accordingly, the pedunculopontine nucleus harbors cholinergic

neurons that project to many regions within the CNS with implications for a broad range of functions.[64] A post-mortem study found abundant Lewy pathology and loss of cholinergic neurons in the pedunculopontine nucleus of subjects with Dementia with Lewy bodies.[63] This may support that loss of cholinergic integrity in the pedunculopontine region could have an impact on non-motor symptoms.

The hypothalamus is involved in the central control of autonomic functions, and affected by Lewy pathology in subjects with Parkinson's disease.[65,66] However, we found no relevant correlations between [^{18}F]FEOBV uptake in this region and measures of subjective or objective autonomic dysfunction.

Non-neuronal and non-parasympathetic cholinergic cells

Alpha-cells in the endocrine pancreas express the vesicular acetylcholine transporter and excrete acetylcholine in a paracrine fashion to affect the beta-cell secretion of insulin.[67] Therefore, lower [^{18}F]FEOBV PET signal in the pancreas may partly be associated with impaired function of alpha-cells. This is interesting because studies have documented an association between type 2 diabetes mellitus and Lewy body disease.[68,69] This link is further supported by post-mortem evidence of phosphorylated alpha-synuclein in beta-cells of subjects with Dementia with Lewy bodies, Parkinson's disease, incidental Lewy body disease, and neurologically intact adults with type 2 diabetes mellitus.[70,71] However, we found no association between pancreatic [^{18}F]FEOBV uptake and measures of blood glucose. Furthermore, stellate cells in the peri-acinar spaces of the exocrine pancreas also express the vesicular acetylcholine transporter.[72] The stellate cells are of neuroectodermal origin and stimulate acinar secretion of enzymes. Collectively, [^{18}F]FEOBV uptake in the pancreas may partly reflect uptake in non-neuronal cholinergic cells.

The enteric nervous system is an extensive network of intrinsic neurons in the gastrointestinal tract, and a large proportion of enteric neurons express the vesicular acetylcholine transporter.[38] Lewy pathology has been found in the enteric nervous system of the colon of subjects with Dementia with Lewy bodies.[1,73] A post-mortem study found no neuronal loss in enteric neurons in the colon or any other segment of the gastrointestinal tract in subjects with Parkinson's disease.[74] The study also reported that the distribution of Lewy pathology in the

colon reflected the parasympathetic innervation from the dorsal motor nucleus of the vagus, not the distribution of enteric neurons. This suggests that degeneration of the enteric neurons may not explain decreased [^{18}F]FEOBV PET uptake in the colon. Further, in the human colon, alpha-synuclein is more frequently present in cholinergic neurons compared to other neuronal types.[75] This suggests that decreased [^{18}F]FEOBV PET uptake in the colon may at least in part reflect parasympathetic denervation.

The majority of subjects with Dementia with Lewy bodies were treated with acetylcholinesterase inhibitors. To our knowledge, no studies have evaluated the effect of acetylcholinesterase inhibitor treatment on [^{18}F]FEOBV binding, the levels of vesicular acetylcholine transporter, or choline acetyltransferase in the human brain. In rodents, most studies using acute or prolonged treatment with acetylcholinesterase inhibitors have not found any effect on [^{18}F]FEOBV binding or levels of vesicular acetylcholine transporter protein.[76-80] Interestingly, some studies have indicated a slight upregulation of vesicular acetylcholine transporter levels.[81,82] Consequently, it is reasonable to conclude that in our studies, treatment with donepezil is unlikely to influence [^{18}F]FEOBV binding or, at the very least, is unlikely to contribute to group differences of diminished [^{18}F]FEOBV uptake in Dementia with Lewy bodies. Nevertheless, the inclusion of subjects on donepezil treatment is a limitation of the study.

A considerable number of eligible subjects declined our invitation to take part of the study (Supplementary Fig.1). The main reason was inability to cope with the study program due to transportation issues, physical limitations, and lack of support from caregivers. Consequently, those that were able to participate may be biased toward better mental and physical function, and more caregiver support. Intentionally, the inclusion of biomarkers in the eligibility criteria may have also skewed the sample towards those with less Alzheimer co-pathology.[83] These biases could have reduced the differences between groups. Despite these limitations, the subjects included in the study had similar age, sex, education, and cognitive profile to that of a larger, autopsy-confirmed cohort of subjects with Dementia with Lewy bodies.[84]

In conclusion, we found impaired cholinergic integrity of the pancreas and colon, which may indicate parasympathetic denervation in subjects with Dementia with Lewy bodies. This

interpretation supports that degeneration in the parasympathetic nervous system, and in particular the dorsal motor nucleus of the vagus, is a characteristic feature of Dementia with Lewy bodies. Longitudinal studies may clarify the rate of progression of cholinergic loss in the pancreas and colon, and the suitability of [^{18}F]FEOBV PET as a biomarker in subjects with prodromal disease. Alternatively, decreased cholinergic integrity of the pancreas may reflect changes in non-neuronal cholinergic cells with implications for understanding the link between Lewy body disease and diabetes. Finally, the study supports that cholinergic denervation in autonomic centers of the brainstem may contribute to autonomic dysfunction in Dementia with Lewy bodies.

Data availability

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

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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 [^{18}F]FEOBV PET uptake in peripheral organs of subjects with Dementia with Lewy bodies and healthy controls. (**A**) Group comparison of [^{18}F]FEOBV PET uptake in the pancreas and colon between subjects with Dementia with Lewy bodies (DLB, n = 23) and healthy controls (HC, n = 21) (Mann-Whitney test). (**B**) Illustrative example of [^{18}F]FEOBV PET uptake in the pancreas of a healthy control (top) and a subject with Dementia with Lewy bodies (bottom). Red colors indicate SUV above 8. (**C**) Whole-body maximum intensity projection presenting the general distribution of tracer and uptake in the liver (**i**), kidney (**ii**), heart (**iii**), and pancreas (**iv**). The spleen (**v**), paraspinal muscle (**vi**), and colon (**vii**) are not visible on the maximum intensity projection image. PET signal is scaled to the approximate level used in the analyses of each organ. (**D**) Group comparisons of [^{18}F]FEOBV PET uptake in peripheral organs with no differences between healthy controls and subjects with Dementia with Lewy bodies. Abbreviations: DLB, Dementia with Lewy bodies; HC, healthy control; ns, not significant. $^{**}P < 0.005$. $^{***}P < 0.001$.

Figure 2 Associations between non-motor symptoms and [^{18}F]FEOBV PET uptake in brainstem regions in Dementia with Lewy bodies. (**A**) Spearman's rank correlations of autonomic symptoms measured on the SCOPA-AUT and non-motor symptoms assessed with the NMSS and [^{18}F]FEOBV PET uptake in the pedunculopontine region (orange) and viscero-sensory-motor nuclei complex (blue) in Dementia with Lewy bodies. The viscero-sensory-motor region corresponds to the location of the solitary nucleus, dorsal motor nucleus of the vagus, hypoglossal nucleus, prepositus, intercalated nucleus, and interpositus. (**B**) 3D image and (**C**) coronal view of the MNI-template with atlas of the pedunculopontine (orange) and viscero-sensory-motor nuclei complex (blue) superimposed. The far-most right image of panel C shows the location of the pedunculopontine region (orange) and viscero-sensory-motor nuclei complex (blue) superimposed on an average [^{18}F]FEOBV PET image of 15 healthy control subjects. Abbreviations: NMSS, Non-Motor Symptoms Scale for Parkinson's Disease; SCOPA-AUT, Scales for Outcomes in Parkinson's Disease – Autonomic Dysfunction. $^{*}P < 0.05$. $^{**}P < 0.005$.

Figure 3 Changes in orthostatic systolic and diastolic blood pressure in Dementia with Lewy bodies and its correlation with [^{18}F]FEOBV PET uptake in the viscero-sensory-

motor nuclei complex of the medulla oblongata. Compared to healthy controls (HC), subjects with Dementia with Lewy bodies (DLB) had larger decreases in (**A**) orthostatic systolic and (**B**) orthostatic diastolic blood pressure (Mann-Whitney test). In Dementia with Lewy bodies, changes in orthostatic blood pressure correlated with [^{18}F]FEOBV uptake in the viscero-sensory-motor nuclei complex. Abbreviations: mmHg, millimeters of mercury; r_s, Spearman's rank correlation coefficient. $^{**}P < 0.005$. $^{****}P < 0.0001$.

Table 1 Demographic and clinical information

	HC (n = 21)	DLB (n = 23)	*P*
Age, years	74.09 ± 6.04	74.37 ± 5.66	0.8757
Sex, male	14 (67%)	19 (83%)	0.3028
Time since diagnosis, years	—	0.36 (0.22–1.17)	—
European ancestry	21 (100%)	23 (100%)	—
Smoking, previous or current	13 (62%)	16 (70%)	0.7520
Smoking, pack years	2 (0–19)	5 (0–23)	0.7800
Alcohol, units/week	6 (3–11)	2 (1–4)	**0.0114**
Education, years	16 (13–21)[a]	14 (13–17)	0.1589
Body mass index	25 (22 – 29)	24 (22 – 25)	0.3787
Parkinson medication	—	7 (30%)[b]	—
Time on Parkinson mediation, years	—	0.67 (0.39–1.31)	—
LEDD, mg	—	350 ± 144	—
Dementia medication	—	19 (83%)[c]	—
Time on dementia medication, years	—	0.29 (0.20–1.09)	—
Core clinical features (I/II/III/IV)	—	2/7/8/6	—
Probable DLB	—	21 (91%)	—
Possible DLB	—	2 (9%)	—
Motor assessment			
UPDRS-III, score	—	40 (27–53)[d]	—
Parkinsonism	—	21 (91%)	—
H&Y 0/I/II/III/IV	—	2/0/20/1/0	—
Biochemical information			
VFG, mmol/L	5.05 (4.70–5.48)[e]	4.95 (4.70–5.20)[f]	0.6052
HbA1c, mmol/mol	37 (36–39)	36 (34–38)[g]	0.1445
Prediabetes[h]	2 (10%)	1 (4%)	0.6085

Demographic and clinical information on 21 healthy control (HC) subjects and 23 patients with Dementia with Lewy bodies (DLB). Continuous variables are reported as mean ± *SD* if normally distributed and median with interquartile range if non-normally distributed. Categorical variables are presented with frequency and percentage. Group comparisons are made with unpaired t-test or Mann-Whitney test for continuous variables and Fisher's exact test for categorical variables. P-values are uncorrected and values below 0.05 marked in bold. Abbreviations: H&Y, Hoehn and Yahr scale; LEDD, levodopa equivalent daily dose; HbA1c, glycated hemoglobin; VFG, venous fasting glucose.

[a]Available for 16 HC subjects.
[b]All DLB patients were treated only with levodopa.
[c]All DLB patients were treated only with cholinesterase inhibitors.
[d]DLB patients were tested while *on* PD medication.
[e]Available for 20 HC subjects.
[f]Available for 20 DLB patients.
[g]Available for 22 DLB patients.
[h]VFG between 5.6 and 6.9 mmol/L or HbA1c between 42 and 47 mmol/mol.

Table 2 Non-motor assessment

	HC (n = 21)	DLB (n = 23)	*P*
MoCA	28 (27–29)	19 (14–21)	**<0.0001**
Systolic blood pressure, mmHg	136±14	132±18	0.3807
Diastolic blood pressure, mmHg	75±7	78±10	0.2544
Supine hypertension[a]	8 (38%)	11 (48%)	0.5567
Orthostatic hypotension	5 (24%)	16 (70%)	**0.0032**
Max systolic drop, mmHg	-4 (-18 – -1)	-31 (-46 – -14)	**<0.0001**
Max diastolic drop, mmHg	3 (-3–8)	-10 (-19 – -4)	**<0.0001**
Odor identification, score	11 (10–14)	5 (2–6)	**<0.0001**
RBDSQ, score	2 (2–4)	11 (7–12)	**<0.0001**
RBD, possible	2 (10%)	20 (87%)	**<0.0001**
MFQ, score	0 (0–1)[b]	3 (2–3)	**<0.0001**
Fluctuations	0 (0%)[b]	13 (57%)	**0.0003**
Hallucinations	0 (0%)[c]	12 (52%)	**0.0004**
FM-100, total error score	58 (29–89)[c]	192 (94–242)[d]	**<0.0001**
GDS-15, score	0 (0–2)[c]	4 (3–7)	**<0.0001**
NMSS			
Cardiovascular/falls, score	0 (0–0)	0 (0–4)	**0.0067**
Sleep/fatigue, score	0 (0–3)	8 (2–18)	**0.0008**
Mood/cognition, score	0 (0–0)	6 (0–15)	**<0.0001**
Perceptual/hallucinations, score	0 (0–0)	2 (0–4)	**<0.0001**
Attention/memory, score	0 (0–1)	14 (6–19)	**<0.0001**
Gastrointestinal, score	0 (0–0)	3 (0–8)	**<0.0001**
Urinary, score	5 (0–10)	16 (7–24)	**0.0010**
Sexual, score	0 (0–2)	2 (0–12)	0.0734
Taste/smell, score	0 (0–0)	3 (0–6)	**0.0002**
Weight change, score	0 (0–0)	0 (0–0)	0.2792
Thermoregulatory, score	0 (0–0)	0 (0–6)	0.0894
Pain, score	0 (0–0)	0 (0–0)	0.3557
Total, score	8 (4–19)	74 (40–100)	**<0.0001**
SCOPA-AUT			
Gastrointestinal, score	0 (0–1)	4 (2–4)	**<0.0001**
Urinary, score	5 (3–8)	8 (4–13)	0.0578
Cardiovascular, score	0 (0–0)	1 (0–3)	**<0.0001**
Thermoregulatory, score	0 (0–1)	2 (1–4)	**0.0056**
Pupillomotor, score	0 (0–0)	1 (0–3)	**0.0143**
Sexual, score	0 (0–1)	3 (0–6)	0.0607
Total, score	8 (5–13)	21 (13–23)	**<0.0001**

Non-motor assessment on 21 healthy control (HC) subjects and 23 patients with Dementia with Lewy bodies (DLB). Continuous variables are reported as mean ± *SD* if normally distributed and median with interquartile range if non-normally distributed. Categorical variables are presented with frequency and percentage. Group comparisons are made with unpaired t-test or Mann-Whitney test for continuous variables and Fisher's exact test for categorical variables. P-values are uncorrected and values below 0.05 marked in bold.

[a]Systolic blood pressure > 140 mmHg or diastolic blood pressure > 90 mmHg.

[b]Available for 15 HC subjects.

[c]Available for 16 HC subjects.

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

Table 3 [^{18}F]FEOBV PET SUV in peripheral regions

Region	HC (*n* = 21)	DLB (*n* = 23)	*P*
Pancreas	7.60 (6.55–8.42)	5.20 (3.36–6.29)	**0.0003**
Colon	1.12 (0.99–1.37)	0.88 (0.75–1.16)	**0.0048**
Muscle	1.57 (1.30–1.73)	1.68 (1.24–2.02)	0.4810
Spleen	2.04 (1.78–2.58)	2.03 (1.67–2.43)	0.8478
Kidneys	1.76 (1.42–1.99)	1.54 (1.30–1.70)	0.0534
Liver	9.25 (7.93–10.45)	9.06 (7.83–10.66)	0.8116
Heart	6.13 (5.35–7.19)	6.17 (5.40–7.74)	0.8027

[^{18}F]FEOBV PET standard uptake values (SUV) in peripheral regions of 21 healthy control (HC) subjects and 23 patients with Dementia with Lewy bodies (DLB). Regions below the dashed line have little or uncertain parasympathetic innervation. Variables are reported as median and interquartile range and group comparisons with the Mann-Whitney test. P-values are uncorrected and values below 0.05 marked in bold.

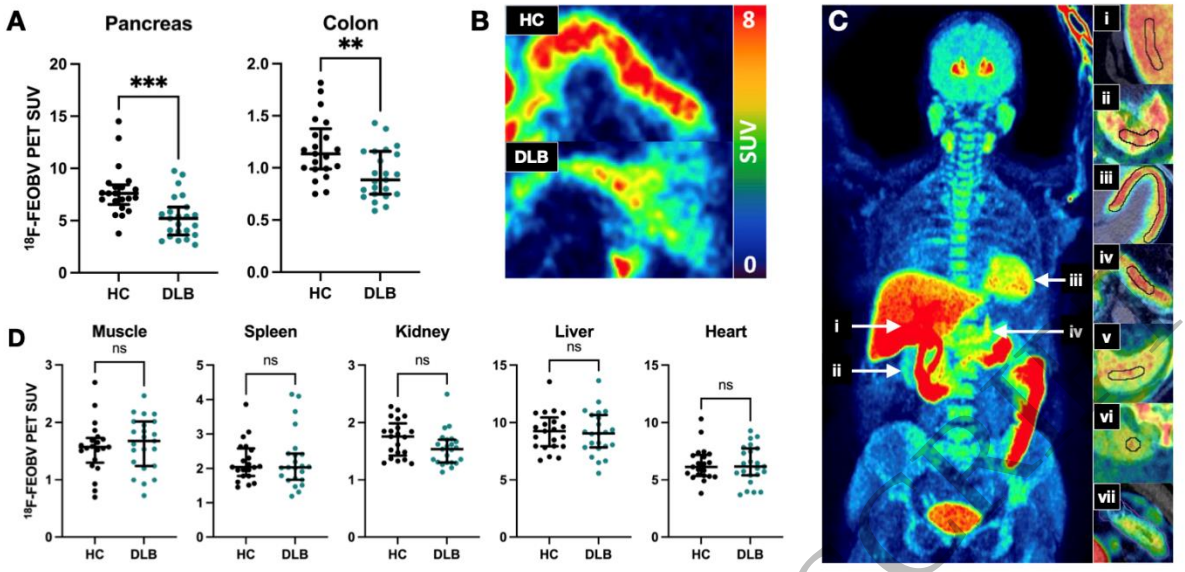

Figure 1
555x271 mm (x DPI)

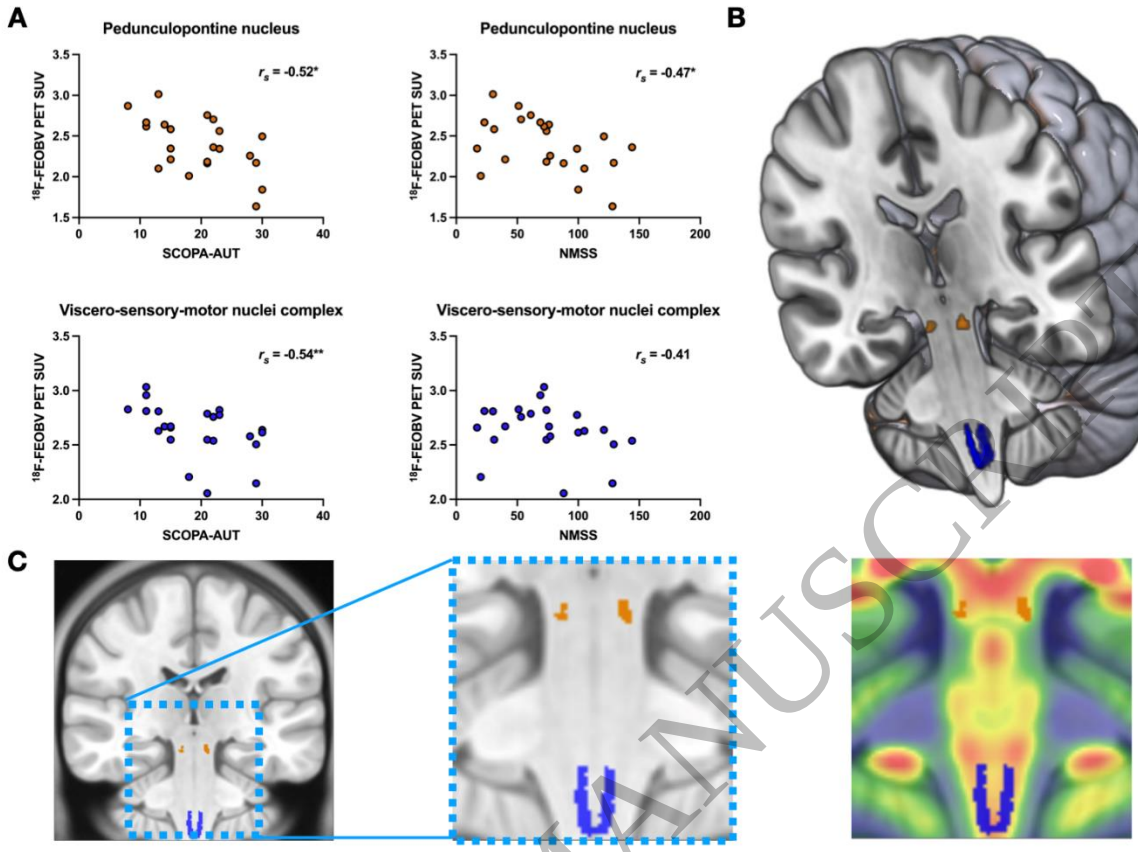

Figure 2
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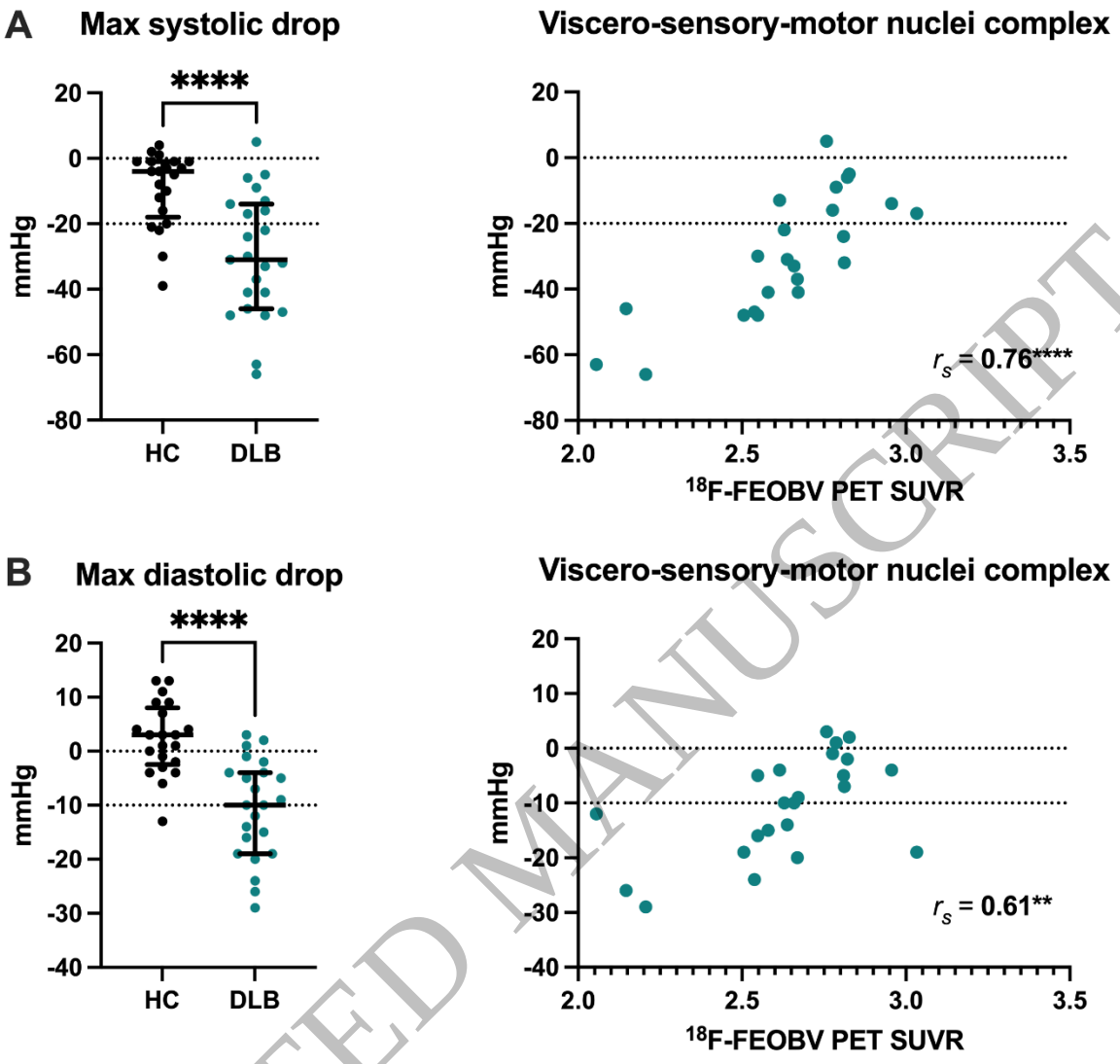

Figure 3
214x202 mm (x DPI)