BRAIN
ORIGINAL ARTICLE

Structural and molecular cholinergic imaging markers of cognitive decline in Parkinson's disease

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Cognitive decline in Parkinson's disease is related to cholinergic system degeneration, which can be assessed *in vivo* using structural MRI markers of basal forebrain volume and PET measures of cortical cholinergic activity. In the present study we aimed to examine the interrelation between basal forebrain degeneration and PET-measured depletion of cortical acetylcholinesterase activity as well as their relative contribution to cognitive impairment in Parkinson's disease.

This cross-sectional study included 143 Parkinson's disease participants without dementia and 52 healthy control participants who underwent structural MRI, PET scanning with ^{11}C-methyl-4-piperidinyl propionate (PMP) as a measure of cortical acetylcholinesterase activity, and a detailed cognitive assessment. Based on the fifth percentile of the overall cortical PMP PET signal from the control group, people with Parkinson's disease were subdivided into a normo-cholinergic ($n = 94$) and a hypo-cholinergic group ($n = 49$). Volumes of functionally defined posterior and anterior basal forebrain subregions were extracted using an established automated MRI volumetry approach based on a stereotactic atlas of cholinergic basal forebrain nuclei. We used Bayesian *t*-tests to compare basal forebrain volumes between controls, and normo- and hypo-cholinergic Parkinson's participants after covarying out age, sex and years of education. Associations between the two cholinergic imaging measures were assessed across all people with Parkinson's disease using Bayesian correlations and their respective relations with performance in different cognitive domains were assessed with Bayesian ANCOVAs. As a specificity analysis, hippocampal volume was added to the analysis.

We found evidence for a reduction of posterior basal forebrain volume in the hypo-cholinergic compared to both normo-cholinergic Parkinson's disease [Bayes factor against the null model (BF_{10}) = 8.2] and control participants (BF_{10} = 6.0), while for the anterior basal forebrain the evidence was inconclusive ($BF_{10} < 3$). In continuous association analyses, posterior basal forebrain volume was significantly associated with cortical PMP PET signal in a temporo-posterior distribution. The combined models for the prediction of cognitive scores showed that both cholinergic markers (posterior basal forebrain volume and cortical PMP PET signal) were independently related to multi-domain cognitive deficits, and were more important predictors for all cognitive scores, including memory scores, than hippocampal volume.

We conclude that degeneration of the posterior basal forebrain in Parkinson's disease is accompanied by functional cortical changes in acetylcholinesterase activity and that both PET and MRI cholinergic imaging markers are independently associated with multi-domain cognitive deficits in Parkinson's disease without dementia. Comparatively, hippocampal atrophy only seems to have minimal involvement in the development of early cognitive impairment in Parkinson's disease.

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Introduction

Parkinson's disease is associated with progressive cognitive decline and a majority of people with Parkinson's disease will eventually develop dementia.[1,2] Cognitive impairment greatly contributes to overall disease burden and worsening of the patient and caregiver's quality of life.[3] While the motor symptoms in Parkinson's disease are mainly attributed to the loss of dopaminergic neurons in the substantia nigra, there is a growing body of evidence for the involvement of cholinergic changes in parkinsonian cognitive decline. The integrity of the cortical and limbic cholinergic system can be investigated *in vivo* by MRI-based assessment of the volume of the cholinergic basal forebrain, which constitutes the main source of cortical cholinergic innervation.[4] Lower basal forebrain volume has been found to be related to impaired cognition across different cognitive domains, in *de novo* as well as more advanced Parkinson's disease.[5,6] A reduction in basal forebrain volume can occur early in the course of the disease and has been found to precede and to be predictive of future development of cognitive impairment in Parkinson's disease.[7–9]

In addition to basal forebrain volume, cortical cholinergic activity can be assessed with PET using tracers that visualize different aspects of cholinergic neurotransmission. Commonly used tracers are ^{11}C-methyl-4-piperidinyl propionate (PMP), which measures cortical acetylcholinesterase activity and ^{18}F-fluoroethoxybenzovesamicol (FEOBV), which binds to the vesicular acetylcholine transporter. Both synaptic acetylcholinesterase activity[10,11] and presynaptic vesicular acetylcholine transporter binding[12] have been found to be decreased in people with Parkinson's disease compared to control subjects and to be related to the severity of cognitive impairment. However, the interrelation of basal forebrain atrophy and cortical cholinergic changes in Parkinson's disease and their relative contributions to cognitive impairment has not been investigated in detail.

Using FEOBV PET, it was recently demonstrated that there is a regionally specific association between basal forebrain volume and cortical cholinergic nerve terminal loss, indicating that degeneration of the basal forebrain in Parkinson's disease is associated with regional loss of functional cholinergic integrity in the cortex.[13]

In the present study we aimed to examine whether similar associations exist between cholinergic basal forebrain degeneration and depletion of cortical acetylcholinesterase activity as measured by ^{11}C-PMP PET, and to further study the relative involvement of these two imaging markers of cholinergic system integrity in cognitive impairment in Parkinson's disease without dementia.

Materials and methods

Participants

This cross-sectional study involved 143 people with Parkinson's disease and 52 healthy control participants.[11] People with Parkinson's disease met the UK Parkinson's Disease Society Brain Bank clinical diagnostic criteria[14] and had no dementia. The majority of Parkinson's disease participants were taking dopaminergic medication, but none were taking cholinesterase inhibitors or anticholinergic medications. All showed evidence of nigrostriatal degeneration on (+)-^{11}C-dihydrotetrabenazine vesicular monoamine transporter type 2 PET except for one participant, where this scan failed due to technical reasons.[11]

All participants underwent a detailed cognitive assessment that has been used previously to characterize cognitive impairment in Parkinson's disease.[15] This assessment covered four cognitive domains with the following tests: (i) memory: California Verbal Learning Test; (ii) executive function: Wechsler Adult Intelligence Scale III Picture Arrangement Test and Delis-Kaplan Executive Function System Sorting Test; (iii) attention: absolute time on the Stroop 1 Test; and (iv) visuospatial function: Benton Judgment of Line Orientation Test. For each domain, a composite Z-score was calculated for each patient based on normative data. Additionally, a global composite cognitive Z-score was calculated by averaging the domain Z-scores. We operationalized mild cognitive impairment (MCI) in the Parkinson's disease group as having a Z-score below 1.5 (i.e. <−1.5 for memory, executive and visuospatial function and >1.5 for attention scores) on at least two of the cognitive domains.[16]

The study was approved by, and study procedures were followed in accordance with the ethical standards of the institutional

review board of the University of Michigan and written informed consent was obtained from each participant.

MRI acquisition

All participants underwent MRI on a 3 T Philips Achieva scanner using an eight-channel head coil and the ISOVOX exam card protocol primarily designed to yield isotropic resolution. A T_1-weighted series of a 3D inversion recovery-prepared turbo-field-echo was performed in the sagittal plane using repetition time = 9.8 ms, echo time = 4.6 ms, inversion time = 1041 ms, turbo factor = 200, single average, field of view = 240 × 200 × 160 mm, acquired matrix = 240 × 200 × 160 slices, and reconstructed to 1 mm isotropic resolution.

MRI analysis

T_1-weighted magnetic resonance images were segmented into grey matter, white matter and CSF, and spatially normalized to MNI space using the CAT12 toolbox in SPM12 (http://www.fil.ion.ucl.ac.uk/spm/). Voxel values of spatially normalized grey matter maps were modulated by the Jacobian determinant of the deformation parameters to preserve the volume present in native space. The volume of the basal forebrain was estimated from the normalized grey matter images by summing up the modulated grey matter values within a consensus region of interest (ROI) based on existing cyto-architectonic maps of basal forebrain cholinergic nuclei in MNI space, which have been derived from combined histology and MRI of post-mortem brains.[17–20] In addition to total basal forebrain volume, we also estimated the volume of two basal forebrain subregions that were identified based on their differential cortical connectivity profile in resting state functional MRI (fMRI) data.[18] In this functionally defined subdivision, the posterior basal forebrain mainly corresponds to the cyto-architectonic subregion of the nucleus basalis of Meynert, while the anterior basal forebrain covers the medial septum and diagonal band of Broca (Fig. 1A).

For comparison, hippocampal volume was determined from the T_1-weighted magnetic resonance images using an analogous automated volumetry approach based on a consensus MNI template of the hippocampus according to the European Alzheimer's Disease Consortium and Alzheimer's Disease Neuroimaging Initiative (EADC-ADNI) Harmonized Protocol.[21] All regional volumes were normalized with respect to total intracranial volume for each participant.

PET acquisition

All Parkinson's disease participants and 11 of the control participants underwent ^{11}C-PMP acetylcholinesterase PET imaging. This was performed in 3D imaging mode using an ECAT HR+ tomograph (Siemens Molecular Imaging), which acquires 63 transaxial slices with slice thickness of 2.4 mm and an intrinsic in-plane resolution of 4.1 mm full-width at half-maximum over a 15.2 cm axial field of view. A NeuroShield head-holder/shielding unit was attached to the patient bed to reduce the contribution of detected photon events originating from the body outside the scanner field of view. Before injection of ^{11}C-PMP a 5-min transition scan was acquired using rotating ^{68}Ge rods for attenuation correction of emission data using the standard vendor-supplied segmentation and projection routines. The ^{11}C-PMP was prepared in high radiochemical purity (>95%) by *N*-^{11}C-methylation of piperidin-4-yl propionate using a previously described method.[22] Dynamic PET scanning was performed for 70 min immediately following a bolus intravenous injection of 555 mega-Becquerel (15 mCi) of ^{11}C-PMP. Emission data were collected in 16 sequential emission scans; four × 30 s, three × 1 min, two × 2.5 min, two × 5 min, and five × 10 min.

PET analysis

All intra-individual image frames were spatially co-registered with a rigid-body transformation to reduce the effects of motion during the scan. Interactive Data Language image analysis software (Research Systems, Boulder, CO) was used to manually trace the caudate nucleus and putamen of each hemisphere on each participant's MRI scan. ^{11}C-PMP hydrolysis rates (k_3) as a measure of acetylcholinesterase activity were then estimated using this striatal volume of interest as the tissue reference for the integral of the precursor delivery.[23]

The Freesurfer software was applied to the magnetic resonance images to define cortical ROIs based on the Desikan-Killiany atlas.[24] The PET images were co-registered with the magnetic resonance images and the mean k_3 within each ROI was extracted as a measure of regional acetylcholinesterase activity. Global cortical acetylcholinesterase activity was calculated as the mean k_3 over all regions.

The Parkinson's disease group was subdivided into a normo-cholinergic ($n = 94$) and a hypo-cholinergic group ($n = 49$) based on the fifth percentile of overall cortical acetylcholinesterase activity of the 11 control participants that underwent ^{11}C-PMP PET scanning as described previously (Fig. 1B).[11]

Statistical analysis

Statistical analyses were conducted in a Bayesian framework using 'Jeffrey's Amazing Statistics Program' (JASP, version 0.17.1) and the BayesFactor (version 0.9.12-4.4) package in R (https://www.r-project.org/). For all models, we report the Bayes factor (BF_{10})[25] to quantify evidence in favour of the alternative over the null hypothesis.[26] Numerical accuracy was established with 10 000 iterations using a Markov Chain Monte Carlo algorithm.

Total, anterior and posterior basal forebrain volumes were compared between the three groups (controls, normo-cholinergic Parkinson's disease, and hypo-cholinergic Parkinson's disease) using Bayesian *t*-tests after covarying out age, sex and years of education. We used the JASP default Cauchy prior. Our alternative hypotheses were that volumes are larger in controls and normo-cholinergic Parkinson's disease compared to hypo-cholinergic Parkinson's disease while we did not expect any difference between controls and normo-cholinergic Parkinson's disease. As a specificity analysis, the same comparison was performed for hippocampal volume, which has been implicated in cognitive impairment in Parkinson's disease in some previous studies,[27,28] but would not be expected to affect cortical cholinergic activity.[29]

In addition to the categorical group comparisons, we conducted continuous association analyses of basal forebrain volume with global and regional cortical acetylcholinesterase activity across the whole Parkinson's disease group using Bayesian correlations with a beta prior distribution for the correlation coefficient with width = 1. The alternative hypothesis was that the correlation does not equal 0.

To test the relative and combinative effects of basal forebrain volume, cortical acetylcholinesterase activity, and hippocampal volume on cognitive scores in the Parkinson's disease participants, Bayesian ANCOVAs were performed including all three imaging metrics as predictors (with covariates for age, sex and years of education) using a beta binomial distribution for the model prior and

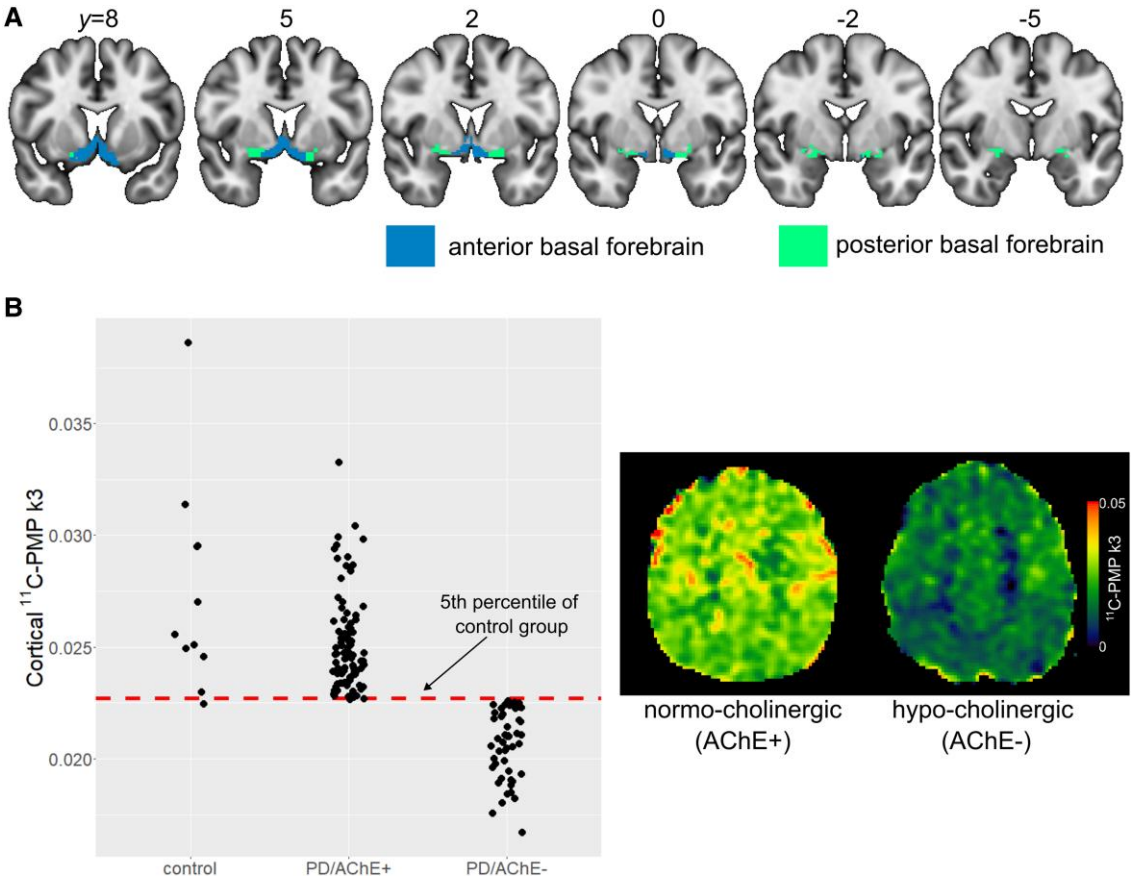

Figure 1 Basal forebrain mask and group stratification. (**A**) Mask of the basal forebrain in MNI space with corticotopically defined subdivisions from Fritz *et al.*[18] (**B**) Division of the Parkinson's disease group according to cholinergic status with images of two representative participants: normo-cholinergic (AChE + PD) = >5th percentile of the control group's cortical acetylcholinesterase activity; hypo-cholinergic (AChE– PD) = < 5th percentile of the control acetylcholinesterase activity. AChE = acetylcholinesterase; PD = Parkinson's disease.

the JASP default JZS prior for coefficients. These ANCOVAs were performed for the composite global score and for each cognitive domain score separately. To test the specificity of associations for cognitive scores, we performed a control analysis using the OFF dopaminergic medications Unified Parkinson's Disease Rating Scale part III (UPDRS-III) motor scores.

Results

Participant characteristic

Table 1 shows demographic and clinical variables for the control, normo-cholinergic and hypo-cholinergic Parkinson's disease groups. There was extreme evidence for an interaction between diagnosis and sex ($BF_{10} = 994.2$), with a markedly higher proportion of male participants in the two Parkinson's disease groups. There was moderate evidence for a group difference in age ($BF_{10} = 7.98$) and Montreal Cognitive Assessment (MoCA) scores ($BF_{10} = 7.19$) with *post hoc* tests revealing that the hypo-cholinergic Parkinson's disease group was older and had lower MoCA scores than the control and normo-cholinergic Parkinson's groups. Furthermore, there was evidence for longer disease duration ($BF_{10} = 19.6$) and higher UPDRS-III scores ($BF_{10} = 6.7$) and Hoehn and Yahr stage ($BF_{10} = 9.2$) in the hypo-cholinergic compared to the normo-cholinergic Parkinson's disease group. Comparison of the cognitive domain Z-scores between the two Parkinson's disease groups showed moderate-to-extreme evidence for lower performance in the hypo-cholinergic compared to the normo-cholinergic group. Nine of the 94 normo-cholinergic Parkinson's disease participants (9.6%) and 20/49 of the hypo-cholinergic Parkinson's disease participants (40.8%) were classified as having MCI.

Association between basal forebrain volume and cholinergic status

For total basal forebrain volume, we found inconclusive evidence for a difference between controls and the hypo-cholinergic Parkinson's disease group ($BF_{+0} = 1.98$), while there was moderate evidence that total basal forebrain volume was reduced in hypo-cholinergic compared to normo-cholinergic Parkinson's disease participants ($BF_{+0} = 4.16$) (Table 2 and Fig. 2A). When considering the basal forebrain sub-regions, it became evident that there was moderate evidence for a reduction of posterior basal forebrain volume in hypo-cholinergic compared to controls ($BF_{+0} = 6.01$) as well as normo-cholinergic Parkinson's disease ($BF_{+0} = 8.17$). In contrast, for the anterior basal forebrain the evidence was inconclusive. As expected, we found moderate evidence for an absence of differences in basal forebrain volumes between controls and normo-cholinergic Parkinson's disease (all $BF_{10} < 0.3$). The evidence for a difference in hippocampal volume based on cholinergic status supported the null hypothesis when comparing controls and hypo-cholinergic Parkinson's disease ($BF_{+0} = 0.11$) while it was inconclusive for the other two comparisons ($BF_{+0} = 0.35$ and $BF_{10} = 1.05$, respectively).

Table 1 Demographics and clinical characteristics

	Controls ($n = 52$)	Normo-cholinergic Parkinson's disease ($n = 94$)	Hypo-cholinergic Parkinson's disease ($n = 49$)	Group differences
Male:female	22:30	65:29	41:8	$BF_{10} = 994.2$[a]
Age	64.0 (12.6)	64.0 (7.2)	69.0 (7.4)	$BF_{10} = 7.98$[b], PD AChE− > Controls, PD AChE+
Education, years	16.2 (2.4)	15.4 (2.9)	15.1 (2.8)	$BF_{10} = 0.41$[b]
MoCA	26.8 (2.3)	26.3 (2.1)	25.2 (3.0)	$BF_{10} = 7.19$[b], PD AChE− < Controls, PD AChE+
Disease duration, years	–	5.3 (3.8)	7.7 (4.8)	$BF_{10} = 19.6$[c]
UPDRS-III	–	30.4 (13.6)	37.4 (14.9)	$BF_{10} = 6.7$°[c]
Hoehn and Yahr stage	–	2.4 (0.5)	2.6 (0.6)	$BF_{10} = 9.2$[c]
LEDD	–	660.3 (544.2)	742.0 (527.7)	$BF_{10} = 0.26$[c]
Z global cognition	–	−0.16 (0.69)	−0.93 (1.24)	$BF_{10} = 2794$[c]
Z memory	–	−0.34 (1.10)	−1.05 (1.25)	$BF_{10} = 40.89$[c]
Z attention[d]	–	−0.07 (0.83)	0.59 (1.50)	$BF_{10} = 32.39$[c]
Z executive	–	−0.31 (0.99)	−1.50 (1.96)	$BF_{10} = 4073$[c]
Z visuospatial	–	−0.07 (0.92)	−0.57 (1.39)	$BF_{10} = 3.56$[c]

Mean (standard deviation). AChE = acetylcholinesterase; BF_{10} = Bayes factor; LEDD = levodopa equivalent daily dose; MoCA = Montreal Cognitive Assessment; PD = Parkinson's disease; UPDRS III = Unified Parkinson's Disease Rating Scale part III (motor score).
[a]Bayesian contingency tables test (independent multinomial).
[b]Bayesian ANOVA.
[c]Bayesian independent samples *t*-test.
[d]Note that attention scores are measured as reaction times and therefore higher *Z*-scores reflect more impairment.

Table 2 Association between cholinergic status and basal forebrain volume

	Controls > AChE − PD	AChE + PD > AChE − PD	Controls ≠ AChE + PD
Total basal forebrain volume	$BF_{+0} = 1.98$	$BF_{+0} = 4.16$	$BF_{10} = 0.19$
Anterior basal forebrain volume	$BF_{+0} = 0.78$	$BF_{+0} = 1.54$	$BF_{10} = 0.19$
Posterior basal forebrain volume	$BF_{+0} = 6.01$	$BF_{+0} = 8.17$	$BF_{10} = 0.20$
Hippocampal volume	$BF_{+0} = 0.11$	$BF_{+0} = 0.35$	$BF_{10} = 1.05$

Results from Bayesian independent sample *t*-tests comparing basal forebrain and hippocampal volumes between control, normo-cholinergic Parkinson, and hypo-cholinergic Parkinson groups after covarying out age, sex and years of education. AChE = acetylcholinesterase; AChE+ = normo-cholinergic; AChE− = hypo-cholinergic; BF_{10} = Bayes factor giving evidence for H_1 over H_0 (for two-sided *t*-test); BF_{+0} = Bayes factor giving evidence for H+ over H_0 (for one-sided *t*-test); PD = Parkinson's disease.

When analysing global cortical ^{11}C-PMP signal as a continuous variable, there was very strong evidence for a positive association between overall cortical acetylcholinesterase activity and posterior basal forebrain volume across all Parkinson's disease participants ($r = 0.29$, $BF_{10} = 46.6$) (Fig. 2B). In contrast, the evidence for an association between overall cortical acetylcholinesterase activity and the volume of the anterior basal forebrain was inconclusive ($r = 0.19$, $BF_{10} = 1.3$). The regional analysis revealed a distinct pattern of associations between posterior basal forebrain volume and cortical acetylcholinesterase activity in different temporal, parietal and occipital regions, as well as the anterior cingulate cortex (Fig. 2C), whereas the only regions that showed a moderate association with anterior basal forebrain volume were left ($BF_{10} = 3.5$) and right ($BF_{10} = 4.8$) anterior cingulate cortex. Further analyses were therefore restricted to the posterior basal forebrain.

Association with cognitive performance

The combined models for the prediction of cognitive scores showed that the two cholinergic markers were more important predictors of cognitive scores than hippocampal volume, but were associated differentially with the different cognitive domains (Table 3 and Fig. 3). The most likely model for the prediction of global cognition contained both cholinergic markers, showed very strong evidence for an association ($BF_{10} = 71.9$), and was 2.5× more likely than the model that also contained hippocampal volume. For memory scores, there was extreme evidence for the models only containing posterior basal forebrain volume ($BF_{10} = 248.4$) or both cholinergic measures ($BF_{10} = 168.3$), and these models were 3.2× and 3.1× more likely than the model that also contained hippocampal volume. For visuospatial function, the model only containing posterior basal forebrain volume ($BF_{10} = 4.78$) was 1.6× more likely than the model that also contained cortical cholinergic activity, and 2.6× more likely than the model also containing hippocampal volume. In contrast, for executive function, the most likely model given the data only contained cortical cholinergic activity (showing strong evidence, $BF_{10} = 10.11$) and was 2.1× more likely than the model that also contained posterior basal forebrain volume, and 3.8× more likely than the model also containing hippocampal volume. For attention scores, the evidence for an association with any of the imaging measures was inconclusive.

The control analysis with UPDRS-III scores did not show evidence for an association with the cholinergic markers or hippocampal volume (Table 3).

A Bayesian *t*-test between people with Parkinson's disease who fulfilled criteria for MCI and those who did not, showed extreme evidence for a decrease in overall cortical acetylcholinesterase activity in the people with MCI ($BF_{10} = 3460$).

Discussion

In this study, we examined associations between two *in vivo* markers of cholinergic deficits—basal forebrain degeneration and depletion of cortical acetylcholinesterase activity—as well as their relative contribution to cognitive impairment in Parkinson's disease without dementia.

We found strong evidence for an association between structural alterations in subcortical cholinergic nuclei within the posterior basal forebrain and functional cortical changes in acetylcholinesterase

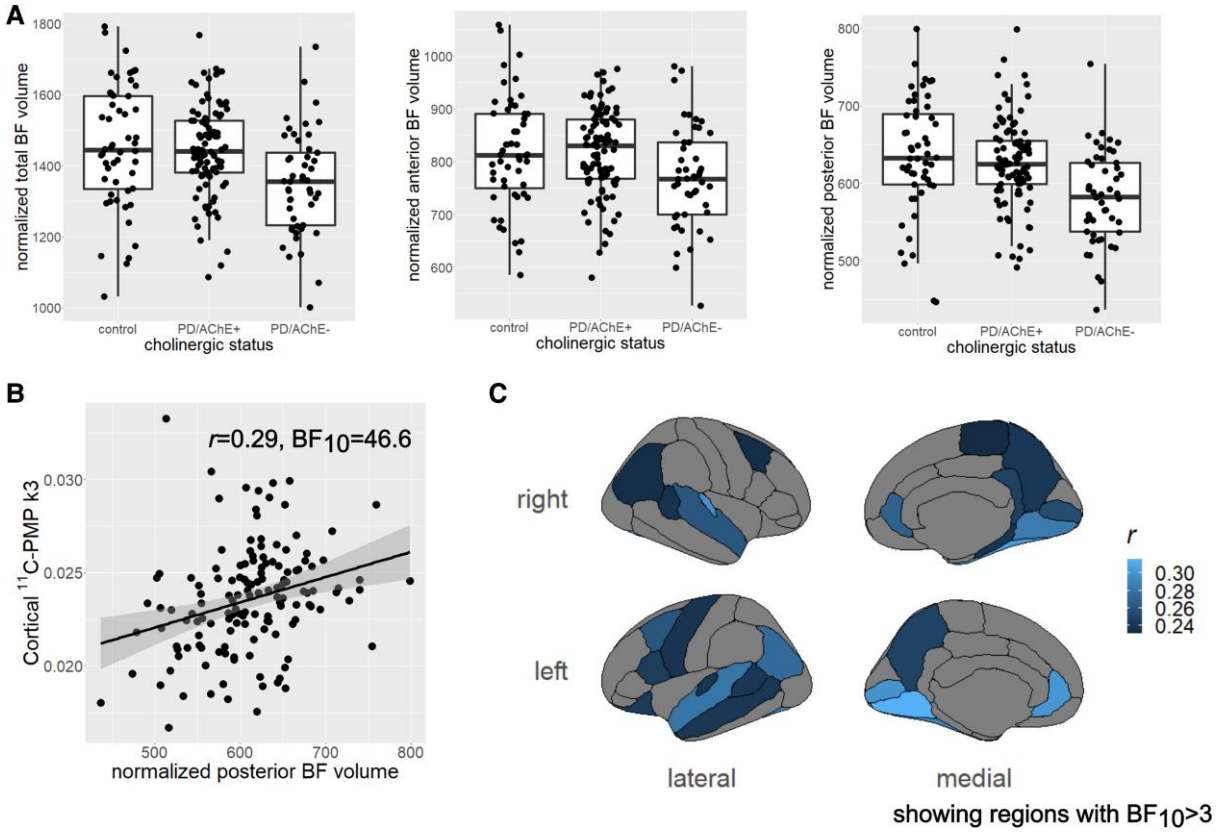

Figure 2 Association between basal forebrain volume and cortical cholinergic activity. (**A**) Group comparison of total and subregional basal forebrain volume (normalized with respect to total intracranial volume) according to cholinergic status. In each box plot the central line corresponds to the sample median, the upper and lower border of the box represent the 25th and 75th percentile, respectively, and the length of the whiskers corresponds to 1.5× the interquartile range. Corresponding results from Bayesian statistical testing are presented in Table 2. (**B**) Correlation between posterior basal forebrain volume and overall cortical acetylcholinesterase activity across all Parkinson's disease participants. (**C**) Associations between posterior basal forebrain volume and cortical acetylcholinesterase activity in 68 regions from the Desikan-Killiany atlas showing all regions with at least moderate evidence for an association (i.e. $BF_{10} > 3$). BF = basal forebrain; BF_{10} = Bayes Factor in favour of H_1 over H_0; PD = Parkinson's disease.

activity. For people with Parkinson's disease whose cortical acetylcholinesterase activity was within the normal range, we found moderate evidence that their posterior basal forebrain volume was not different from controls. In contrast, those participants who were categorized as hypo-cholinergic based on cortical ^{11}C-PMP binding showed evidence for degeneration of the posterior basal forebrain not only compared to controls, but also in comparison to the normo-cholinergic Parkinson's disease group. Additionally, across the whole patient group, smaller posterior basal forebrain volume was associated with reduced cortical acetylcholinesterase activity. These *in vivo* findings confirm post-mortem histological investigations that have suggested that the cortical acetylcholinesterase deficit observed in Parkinson's disease is related to the degeneration of cholinergic neurons in the basal forebrain.[30,31] The regional analysis revealed a largely symmetric pattern with the strongest associations for bilateral occipital, temporal and parietal regions. This is consistent with previous cholinergic PET studies that have reported an early vulnerability of occipital and temporo-parietal cortices in Parkinson's disease[32–34] and a posterior-to-anterior gradient of cholinergic denervation along the disease course from Parkinson's disease to Parkinson's disease dementia.[35,36] The finding that it was mainly the volume of the posterior part of the basal forebrain that was related to cortical cholinergic activity is in keeping with the known anatomic projections of the different basal forebrain nuclei: the posterior basal forebrain, which mainly corresponds to the nucleus basalis of Meynert, provides the majority of cholinergic input to the neocortex.[4,37] By contrast, the anterior part of the basal forebrain (corresponding to cholinergic cell groups Ch1–Ch3) projects mainly to the hippocampus, hypothalamus and olfactory bulb, and consequently we did not find evidence for a reduction of its volume in Parkinson's disease participants with cortical cholinergic deficits.

A recent study reports a similar analysis in Parkinson's disease using FEOBV PET, a novel cholinergic tracer that binds to the vesicular acetylcholine transporter.[13] Associations between cortical cholinergic activity and basal forebrain volume could also be observed in this study; however, the regional patterns only partly overlapped with the present results. In line with our findings, basal forebrain volume was robustly correlated with FEOBV PET binding in temporal regions.[13] However, in contrast to the present findings and in contrast to their *a priori* hypothesis, Ray *et al.*[13] did not find strong associations between basal forebrain volume and cholinergic activity in posterior regions. The authors speculated that this might be due to statistical floor effects with respect to cholinergic terminal loss in posterior areas, which does not seem to affect the cortical measures of acetylcholinesterase activity employed in our study.

Despite the fact that we found a clear association between posterior basal forebrain volume and cortical acetylcholinesterase activity, the regression analyses with cognitive scores showed that

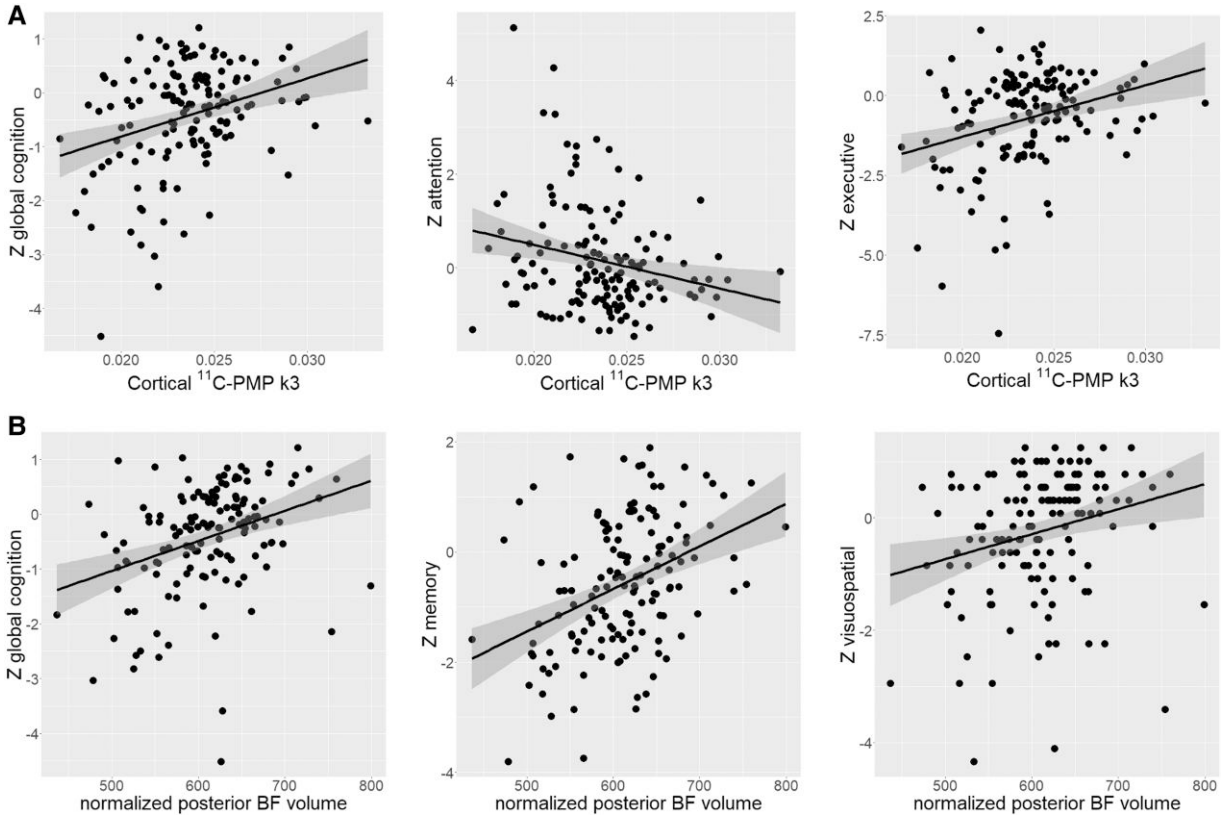

Figure 3 Association with cognitive scores. Association of (**A**) cortical acetylcholinesterase activity and (**B**) posterior basal forebrain volume with different cognitive domain scores in the Parkinson's disease participants, showing all comparisons for which there is at least moderate evidence for the presence of an association after controlling for age, sex and years of education ($BF_{10} > 3$). Corresponding results from Bayesian statistical testing can be found in Table 3. BF = basal forebrain.

the two cholinergic markers were not interchangeable when considering associations with cognition. Both markers independently contributed to performance in different cognitive domains, including memory, attention, executive function and visuospatial function. This indicates that the two cholinergic imaging modalities might reflect partly different aspects of cholinergic degeneration that both contribute to cognitive function in Parkinson's disease. Previous studies have shown an involvement in Parkinson's disease-related cognitive decline separately for basal forebrain volume[5–8] and different cortical cholinergic PET tracers.[10,12,34,38] In the present study, we extend these findings by showing that both cholinergic markers are relevant to the study of cognition in Parkinson's disease and can provide complementary information.

In contrast to the strong evidence for an involvement of the cholinergic system in cognitive impairment in Parkinson's disease, we found evidence for an absence of an association between cognitive performance and hippocampal volume. In particular, in the combined regression model it became evident that even for memory function, the cholinergic markers were more strongly related to cognitive performance than hippocampal volume. This confirms findings from a recent study in an independent cohort that found basal forebrain volume to be more strongly related to cognitive impairment in Parkinson's disease than hippocampal volume.[6] With the help of the Bayesian framework employed in the present study, we could strengthen these results by providing concrete evidence for an absence of an association between hippocampal volume and cognition. A recent meta-analysis found hippocampal atrophy in people with Parkinson's disease with dementia, but not in people with MCI, indicating that hippocampal degeneration might become more important only later in the course of Parkinson's-related cognitive impairment.[39] It has also been suggested that microstructural properties as measured by diffusion tensor imaging might be more sensitive to cognition-relevant changes within the hippocampus than frank atrophy.[6,40] Nevertheless, our results indicate that compared to cholinergic changes, hippocampal atrophy only plays a minor role in the development of cognitive impairment during the pre-dementia stage of Parkinson's disease.

Limitations

A limitation of the ^{11}C-PMP tracer is that it does not allow accurate assessment of acetylcholinesterase activity in regions with high levels of acetylcholinesterase, such as the striatum and cerebellum. The analyses in the present study were therefore confined to cortical regions. Additionally, for this tracer the contribution of pre- versus post-synaptic signal is unknown and FEOBV PET has more recently been developed as a specific presynaptic tracer. It should also be noted that there is high collinearity between anterior and posterior basal forebrain volume due to their close spatial proximity. This partly precludes conclusions about the specificity of effects for the posterior basal forebrain. Similarly, it is difficult to control for the influence of more general degenerative processes due to the fact that basal forebrain volume and overall grey matter volume tend to be highly correlated. We have attempted to partly address

Table 3 Combined model for prediction of cognitive scores

Model	P(M)	P(M|data)	BF_M	BF_{10}	Error %	Evidence for H_1
Global cognition						–
Null model	0.25	0.015	0.048	1.0	–	–
pBF volume + cortical AChE	0.083	0.35	6.74	71.9	1.47	Very strong
pBF volume + cortical AChE + hippo volume	0.25	0.42	1.73	29.1	1.57	Strong
pBF volume	0.083	0.088	0.99	18.1	1.36	Strong
Cortical AChE	0.083	0.059	0.64	12.3	1.36	Strong
pBF volume + hippo volume	0.083	0.050	0.53	10.3	1.33	Strong
Cortical AChE + hippo volume	0.083	0.018	0.18	3.63	1.48	Moderate
Hippo volume	0.083	0.001	0.012	0.26	1.33	Moderate for H_0
Memory						–
Null model	0.25	0.005	0.013	1.0	–	–
pBF volume	0.083	0.38	5.71	248.4	1.29	Extreme
pBF volume + cortical AChE	0.083	0.25	3.07	168.3	1.55	Extreme
pBF volume + hippo volume	0.083	0.12	1.13	76.7	1.20	Very strong
pBF volume + cortical AChE + hippo volume	0.25	0.24	0.75	53.7	1.73	Very strong
Cortical AChE + hippo volume	0.083	0.003	0.03	1.99	1.76	Inconclusive
Cortical AChE	0.083	0.003	0.02	1.80	1.60	Inconclusive
Hippo volume	0.083	0.001	0.01	0.89	5.14	Inconclusive
Attention						–
Null model	0.25	0.29	1.16	1.0	–	–
Cortical AChE	0.083	0.20	2.91	2.07	1.68	Inconclusive
pBF volume + cortical AChE	0.083	0.11	1.29	1.10	1.85	Inconclusive
pBF volume	0.083	0.074	0.85	0.76	1.69	Inconclusive
Cortical AChE + hippo volume	0.083	0.066	0.75	0.68	1.86	Inconclusive
pBF volume + cortical AChE + hippo volume	0.25	0.174	0.64	0.59	2.07	Inconclusive
pBF volume + hippo volume	0.083	0.054	0.59	0.55	1.46	Inconclusive
Hippo volume	0.083	0.030	0.32	0.31	2.40	Inconclusive
Executive						–
Null model	0.25	0.10	0.33	1.0	–	–
Cortical AChE	0.083	0.35	5.73	10.11	1.45	Strong
pBF volume + cortical AChE	0.083	0.17	1.96	4.92	1.58	Moderate
Cortical AChE + hippo volume	0.083	0.092	0.94	2.65	1.57	Inconclusive
pBF volume + cortical AChE + hippo volume	0.25	0.22	0.73	2.13	1.69	Inconclusive
pBF volume	0.083	0.03	0.28	0.87	1.37	Inconclusive
pBF volume + hippo volume	0.083	0.019	0.17	0.54	1.49	Inconclusive
Hippo volume	0.083	0.009	0.08	0.25	1.34	Moderate for H_0
Visuospatial						–
Null model	0.25	0.17	0.54	1.0	–	–
pBF volume	0.083	0.27	3.81	4.78	1.27	Moderate
pBF volume + cortical AChE	0.083	0.17	2.02	3.04	1.60	Moderate
pBF volume + hippo volume	0.083	0.10	1.08	1.81	1.66	Inconclusive
Cortical AChE	0.083	0.064	0.64	1.14	1.49	Inconclusive
pBF volume + cortical AChE + hippo volume	0.25	0.19	0.62	1.10	1.79	Inconclusive
Cortical AChE + hippo volume	0.083	0.022	0.21	0.40	1.68	Inconclusive
Hippo volume	0.083	0.017	0.16	0.30	3.65	Moderate for H_0
UPDRS-III						
Null model	0.25	0.44	1.99	1.0	–	–
pBF volume	0.083	0.19	2.76	1.28	1.67	Inconclusive
pBF volume + cortical AChE	0.083	0.077	0.93	0.53	1.87	Inconclusive
Cortical AChE	0.083	0.074	0.89	0.51	1.70	Inconclusive
pBF volume + hippo volume	0.083	0.063	0.74	0.43	13.8	Inconclusive
Hippo volume	0.083	0.056	0.65	0.38	1.82	Inconclusive
Cortical AChE + hippo volume	0.083	0.031	0.34	0.21	1.96	Moderate for H_0
pBF volume + cortical AChE + hippo volume	0.25	0.075	0.28	0.17	2.13	Moderate for H_0

Results from Bayesian ANCOVAs across all Parkinson's disease patients with the different cognitive scores as dependent variable and all three imaging measure (cortical acetylcholinesterase activity, posterior basal forebrain volume, hippocampal volume) as predictors including covariates for age, sex and years of education. Note: all models (including the null model) include age, sex and years of education. AChE = acetylcholinesterase; BF_{10} = Bayes factor in favour of H_1 over H_0; BF_M = degree to which the data have changed the prior model odds; hippo = hippocampus; error % = numerical stability of BF_{10} over 10 000 Markov Chain Monte Carlo iterations; pBF = posterior basal forebrain; PD = Parkinson's disease; P(M) = model's prior probability; P(M|data) = model's posterior probability after observing the data; UPDRS-III = Unified Parkinson's Disease Rating Scale III (motor scores).

this issue by including control analyses of hippocampal volume; however, this remains as a general limitation of studies of basal forebrain volume. Another potential limitation is the fact that the Parkinson's disease group was predominantly male, which is an inherent aspect of many Parkinson's disease studies due to its higher prevalence in males.[41]

Conclusion

We have shown that degeneration of the posterior basal forebrain in Parkinson's disease is accompanied by functional changes in cortical cholinergic neurotransmission and that structural and molecular imaging markers of cholinergic system integrity are independently associated with multi-domain cognitive deficits in Parkinson's disease without dementia. In comparison to these cholinergic changes, hippocampal atrophy only seems to have relatively minimal involvement in the development of early cognitive impairment in Parkinson's disease.

Data availability

The data are available from the corresponding authors upon reasonable request.

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Competing interests

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