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Involvement of the basal forebrain and hippocampus in memory deficits in Parkinson's disease

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ABSTRACT

Introduction: Magnetic resonance imaging (MRI)-determined atrophy of the nucleus basalis of Meynert (Ch4) predicts cognitive decline in Parkinson's disease (PD). However, interactions with other brain regions causing the decline remain unclear. This study aimed to describe how MRI-determined Ch4 atrophy leads to cognitive decline in patients with PD.

Methods: We evaluated 137 patients with PD and 39 healthy controls using neuropsychological examinations, MRI, and ^{123}I-ioflupane single-photon emission computed tomography. First, we explored brain areas with regional gray matter loss correlated with Ch4 volume reduction using voxel-based morphometry (VBM). We then assessed the correlation between Ch4 volume reduction and cognitive impairments in PD using partial correlation coefficients (r_{par}). Finally, we examined whether the regional gray matter loss mediated the association between Ch4 volume reduction and cognitive impairments using mediation analysis.

Results: Our PD cohort was "advanced-stage enriched." VBM analyses revealed that Ch4 volume loss was correlated with volume reduction in the medial temporal lobe in PD ($P < 0.05$, family-wise error corrected, >29 voxels). Ch4 volume reduction was significantly correlated with verbal memory deficits in PD when adjusted for age, sex, total brain volume, and ^{123}I-ioflupane uptake in the caudate ($r_{par} = 0.28$, $P < 0.001$). The mediation analysis revealed that the hippocampus mediated the effects of Ch4 volumes on verbal memory (average causal mediation effect = 0.013, 95 % CI = 0.006–0.020, $P < 0.001$).

Conclusion: Particularly in advanced-stage PD, Ch4 atrophy was associated with medial temporal lobe atrophy, which played an intermediary role in the relationship between Ch4 atrophy and verbal memory impairments.

1. Introduction

Parkinson's disease (PD) has various non-motor symptoms, including cognitive decline. A previous longitudinal study has shown that Mild Cognitive Impairment (MCI) is a harbinger of dementia in PD [1]. The timing, profile, and rate of cognitive decline vary widely among individuals with PD [2]. In PD, major motor symptoms such as bradykinesia and rigidity result from dopaminergic neural dysfunction, while cognitive impairments have been attributed to multiple neurotransmitter dysfunctions including choline, serotonin, and noradrenaline and to cortical and subcortical neurodegeneration [3]. Despite being common and important, the pathophysiology of cognitive decline in PD is still not fully understood.

Recently, several Magnetic Resonance Imaging (MRI) studies indicated that the volume reduction of the nucleus basalis of Meynert (NBM) (Ch4) predicted the development of cognitive impairment in patients

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with PD [4]. Ch4 is the main cholinergic input to the amygdala and entire cortex and constitutes part of the Basal Forebrain (BF). The BF also involves the medial septum and vertical limb of the diagonal band (Ch1-2) and horizontal limb of the diagonal band nucleus (Ch3), which provide cholinergic projections to the hippocampus and olfactory bulb, respectively [5]. Importantly, a marked neuronal loss at Ch4 was observed in PD patients with Dementia (PDD) [6]. Reduced cortical acetylcholinesterase (AchE) activity was found using Positron Emission Tomography (PET) in patients with PD with cognitive decline [7]. Notably, a recent study investigating the interrelation between BF degeneration and PET-measured depletion of cortical AChE activity in PD suggested that there is an association between the volume of the posterior BF, which mainly corresponds to Ch4, and cortical AChE activity in the temporal, parietal, and occipital regions, as well as the anterior cingulate cortex [8].

Based on these studies, the association of MRI-determined Ch4 atrophy with cognitive impairment is often attributed to Ch4-associated cholinergic dysfunction. However, neural deficits other than cholinergic dysfunction may also contribute to cognitive impairments [9]. In Alzheimer's disease (AD), Ch4 atrophy predicted and preceded the memory impairments, and entorhinal cortex atrophy and memory impairments were observed when pathological changes spread from Ch4 to the entorhinal cortex rather than being limited to Ch4 atrophy alone [10]. In PD, the cognitive domains associated with Ch4 atrophy differ between reports [4,11]. In fact, the Braak stage, an α-synuclein-based pathological classification in sporadic PD, showed that the BF, the second sector of the Ammon's horn (part of the hippocampus), the amygdala, and the anteromedial temporal mesocortex demonstrated α-synuclein pathology at the same stage [12]. These regions are distinct from those involved in PET-measured depletion of cortical AChE activity associated with the degeneration of the posterior BF. Therefore, in PD, not only the cholinergic deficit but also synergistic disturbances in multiple other brain regions might be crucial in cognitive decline.

In this study, we aimed to examine the intermediary role of Ch4-related gray matter atrophy in the relationship between Ch4 atrophy and cognitive deficits by conducting causal mediation analyses. We also used ^{123}I-ioflupane Single-Photon Emission Computed Tomography (SPECT) to examine the correlation of cognitive impairment with dopaminergic dysfunction, which is difficult to assess using brain morphometry alone. We examined whether Ch4-related gray matter atrophy and cognitive deficits could be specific to Ch4 volumes by conducting the same analyses on other regions of the BF, including Ch1-2 and Ch3.

2. Methods

2.1. Participants

Overall, 172 patients with PD from the Kyoto University Hospital were invited between September 2016 and May 2019. Patients were diagnosed according to the Movement Disorder Society (MDS) clinical diagnostic criteria for PD [13]. Of these, 137 patients with PD completed a comprehensive neuropsychological battery, brain MRI, and ^{123}I-ioflupane SPECT and were included (Fig. 1). We recruited healthy participants through outreach efforts for a healthy community. Fifty-nine healthy participants were also approached, of whom 39 with Montreal Cognitive Assessment Japanese version (MoCA-J) score above 25 were judged cognitively normal Healthy Controls (HCs). The HCs subsequently underwent the neuropsychological battery and brain MRI. Time interval between the brain MRI, ^{123}I-ioflupane SPECT, and neuropsychological tests for patients with PD, as well as the brain MRI and neuropsychological tests for HCs, were detailed in Supplementary Table S1. Participants were excluded if they had a history of neurological or psychiatric disorders other than PD. Each patient with PD was classified as being Cognitively Normal (CN) or having MCI or dementia following the diagnostic criteria for PD-MCI level 1 and dementia

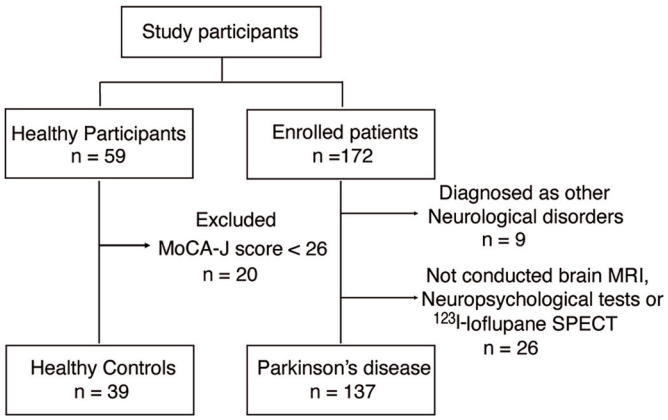

Fig. 1. Study participants. The characteristics of healthy controls and patients with Parkinson's disease. MoCA-J, Japanese version of the Montreal Cognitive Assessment; SPECT, Single-Photon Emission Computed Tomography.

associated with PD [14,15]. All participants or their designated caregivers provided written informed consent in accordance with the dictates of the Ethics Committee, Kyoto University Graduate School and Faculty of Medicine (R0494). The study was conducted in accordance with the tenets of the Declaration of Helsinki and its subsequent amendments.

2.2. Clinical behavioral measures

A neuropsychological battery was conducted in five cognitive domains [14]. Attention and working memory were assessed using the Trail Making Test (TMT)-A and Symbol Digit Modalities Test (SDMT). Executive function was evaluated using the TMT-B. Language function was measured using category fluency. Memory function was estimated using delayed recall of the Hopkins Verbal Learning Test-Revised (HVLT-R) and delayed recall of the Ray–Osterrieth Complex Figure Test (RCFT). Visuospatial function was judged using Benton's Judgement of Line Orientation (JLO). Global functioning was also evaluated using MoCA-J. Patients received the neuropsychological examinations in a drug-on state.

The severity of parkinsonian motor symptom was examined using the Hoehn and Yahr (HY) stage and MDS Unified Parkinson's Disease Rating Scale (MDS-UPDRS) Part III score [13]. We defined the drug-off state as the condition after withdrawing all anti-Parkinsonian medications for at least 12 h. We calculated the Levodopa Equivalent Daily Dose (LEDD) [16].

2.3. MRI data acquisition and preprocessing

MRI scans were acquired using the 3-T Magnetom Skyra system with a 32-channel head coil (Siemens Healthineers, Erlangen, Germany). Whole brain T1-weighted anatomical images were acquired using a magnetization prepared rapid gradient echo sequence with the following parameters: repetition time, 1900 ms; echo time, 2.58 ms; inversion time, 900 ms; flip angle, 9°; field of view, 230 × 230 mm; slices 192; voxel size, 0.9 × 0.9 × 0.9 mm. Conventional T2-and T2*-weighted images were acquired to confirm the absence of structural MRI abnormalities associated with other causes of parkinsonism.

We applied Voxel-Based Morphometry (VBM) [17] using SPM12 (http://www.fil.ion.ucl.ac.uk/spm/software/spm12/) implemented in MATLAB R2018a (MathWorks, Natick, MA). T1-weighted anatomical images were segmented into Gray Matter (GM), White Matter (WM), and Cerebrospinal Fluid (CSF). The resulting GM images in the native space were high-dimensionally registered to each group-specific template and normalized to the Montreal Neurological Institute (MNI) space using Diffeomorphic Anatomical Registration Through Exponentiated Lie

Algebra [18]. The voxel values of GM images were modulated for volumetric changes and the total amount of GM volume was preserved. The modulated images were smoothed with a Gaussian kernel with a full width at half maxima of 8 mm. Total Brain Volume (TBV) was computed by summing the volume values of GM and WM.

2.4. Probabilistic maps of the BF

Regions Of Interest (ROIs) of Ch1-2, Ch3, and Ch4 were identified by using the probabilistic anatomical maps that were created from microscopic histological post-mortem analysis of 10 brains [19]. The anatomical Volumes Of Interest (VOIs) were defined as voxels with values above 50 % probability of these maps. GM volume within each region was calculated using the modulated GM images.

^{123}I-ioflupane SPECT data acquisition and preprocessing.

Scans were obtained using the Infinia GE Healthcare system (GE Healthcare, Waukesha, WI), and 65-slice images were acquired. Details regarding the data acquisition and preprocessing of ^{123}I-ioflupane SPECT data were described in Supplementary Text S1.

2.5. Statistical analysis

When comparing the results of the neuropsychological tests, the scores obtained from both HCs and patients with PD were converted to Z scores adjusted for age, sex, and education years using a linear regression model. The distribution of values between the two groups was compared using two-sample t-tests for continuous variables. Statistical analyses were conducted using R (R Foundation, Vienna, Austria), with the threshold for significance set at $P < 0.05$.

2.6. VBM analysis

We explored each brain area showing a correlation of regional GM loss with Ch1-2, Ch3, and Ch4 volume reduction with age, sex and TBV as nuisance covariates. The significant threshold was set at $P < 0.05$ family-wise error (FWE) corrected with an extent threshold of k of 29 (= expected voxels per cluster). These brain areas were then parceled into regions using the Neuromorphometrics atlas in SPM12 (http://www.neuromorphometrics.com/). We calculated GM volume within each region using the modulated GM images.

2.7. Analysis for cognitive associations with Ch4 volumes and caudate dopaminergic terminal

After assessing correlations between Ch4 volumes and age, other BF volumes (Ch1-2 and Ch3), and the caudate uptake ratio, as detailed in Supplementary Text 1, the relationships between Ch4 volumes and each cognitive performance were evaluated using Pearson's partial correlations controlling for age, sex, TBV, and the caudate uptake ratio. The relationships between the caudate uptake ratio and each cognitive performance were also assessed controlling for age, sex, and the Ch4 volumes. In this analysis, to account for multiple comparisons, we used Bonferroni correction. The original alpha level of $\alpha = 0.05$ was divided by the number of tests conducted ($n = 16$). Therefore, $P < 0.0031$ (0.05/16) was considered statistically significant. We also assessed associations between other BF volumes and cognitive abilities using Pearson's partial correlation, controlling for age, sex, TBV, and the caudate uptake ratio.

2.8. Mediation analysis with Ch4 volumes, cognitive data, and each region correlated with Ch4 volumes

Causal mediation analyses were performed to examine the intermediary role of individual regional GM atrophy in the relationship between Ch4 atrophy and cognitive deficits, using the "mediation" R package. First, we constructed multiple linear regression models for analyses involving Ch4 volumes, each region, and cognitive data controlling for age, sex, TBV, and the caudate uptake ratio. Next, we computed the Average Direct Effect (ADE) and Average Causal Mediation Effect (ACME), reflecting the direct and indirect effects of Ch4 volumes on cognitive data. Both measurements (ACME and ADE) were estimated by models using the quasi-Bayesian Monte Carlo method with 1000 simulations ($P < 0.05$) [20]. We also estimated the mediated proportion of each Ch4-correlated region. If the ACME in specific regions was significant, we also performed complementary mediation analysis between those specific regions and Ch4-related cognitive data, with Ch4 volume as the mediator. In addition, to justify the mediation result, we examined regions of GM volume reduction associated with cognitive tests, using age, sex, and TBV as nuisance covariates, with VBM analysis. The significance threshold was set at $P < 0.05$, FWE corrected, with an extent threshold of $k = 29$ (equal to the expected voxels per cluster). Finally, we performed Pearson's partial correlations between Ch4 volumes and cognitive data, adding regions having significant mediation effects.

2.9. Subgroup analysis

We divided patients with PD into two groups: Cognitively Normal (PD-CN) and MCI or Dementia (PD-MCI/PDD). We then performed all the same analyses as described above separately for each group (i.e., VBM, Pearson's partial correlation, and mediation analyses).

3. Results

3.1. Clinical characteristics

A total of 137 patients with PD and 39 HCs were included. Clinical characteristics of the participants are reported in Table 1. Among patients with PD, 65, 62, and 10 individuals were classified as having CN, MCI, and dementia, respectively. In our cohort, the PD group consisted of a high proportion of individuals with long disease duration and low global cognition; hence, it was characterized as an "advanced-stage enriched" cohort. Ch1-2 and Ch4 volumes of patients with PD were smaller than those of HCs. The TBV of patients with PD was not significantly different from that of HCs (Supplementary Table S2).

3.2. Brain areas of regional GM volume loss correlated with BF volume reduction

In all patients with PD, Ch4 volume reduction was correlated with GM volume loss in the bilateral amygdala, and parts of the bilateral entorhinal cortex, hippocampus, fusiform gyrus, middle and inferior temporal gyri, middle and posterior cingulate gyri, and left precentral gyrus ($P < 0.05$ FWE-corrected, expected voxels per cluster >29) (Fig. 2a). Supplementary Table S3 presents details of respective cluster size and MNI coordinates of the significant areas. In contrast, we did not observe widespread correlations between Ch1-2 or Ch3 volumes and bilateral medial temporal lobe regions, including the bilateral hippocampus and bilateral entorhinal cortex, as we did with Ch4 volumes (Supplementary Figs. S1a and b, and Supplementary Tables S4 and S5).

3.3. Cognitive associations of Ch4 volumes with other BF regions and caudate dopaminergic terminals

In patients with PD, Ch4 volumes were related to age ($r = -0.49$, 95 % CI = −0.60 to −0.35, $P < 0.001$). Ch4 volumes were also associated with Ch1-2 volumes ($r = 0.62$, 95 % CI = 0.50 to 0.71, $P < 0.001$) and Ch3 volumes ($r = 0.71$, 95 % CI = 0.62 to 0.79, $P < 0.001$) after adjusting for age, sex, and TBV, and with TBV ($r = 0.75$, 95 % CI = 0.67 to 0.82, $P < 0.001$) after adjusting for age and sex. After controlling for age, sex, and TBV, Ch4 volumes were also related to the caudate uptake ratio ($r = 0.21$, 95 % CI = 0.04 to 0.36, $P = 0.017$).

We therefore next examined whether Ch4 volume and the caudate

Table 1
Clinical characteristics of patients with Parkinson's disease and healthy controls.

	PD (n = 137) Mean (SD)	HC (n = 39) Mean (SD)	P value[a]
Age	63.5 (9.4)	56.6 (9.1)	P < 0.001
Male/Female (n)	61/76	10/29	
Education years	14.1 (2.3)	13.8 (2.2)	P = 0.51
CN/MCI/Dementia (n)	65/62/10		
Disease duration (years)	9.3 (4.5)		
Hoehn and Yahr scale (off)[b] 1/2/3/4/5 (n)	6/51/26/19/24		
MDS-UPDRS Part III score (off)[b]	41.4 (16.6)		
Hoehn and Yahr scale (on)[c] 1/2/3/4/5 (n)	11/97/24/2/0		
MDS-UPDRS Part III score (on)[c]	20.6 (11.2)		
LEDD	680 (397)		
MoCA-J score	24.7 (3.6)	28.3 (1.4)	–
MoCA-J (Z score)	−0.18 (1.01)	0.64 (0.60)	P < 0.001
SDMT	43.4 (13.6)	58.4 (9.0)	–
SDMT (Z score)	−0.18 (0.99)	0.64 (0.75)	P < 0.001
TMT-A (sec)[d]	46.7 (33.2)	27.7 (8.0)	–
TMT-A (Z score)[d]	0.09 (1.10)	−0.30 (0.44)	P = 0.001
TMT-B (sec)[d]	128.5 (106.2)	65.0 (18.3)	–
TMT-B (Z score)[d]	0.12 (1.08)	−0.42 (0.44)	P < 0.001
JLO	22.4 (5.4)	24.2 (3.4)	–
JLO (Z score)	−0.04 (1.06)	0.14 (0.77)	P = 0.25
Category fluency test	18.5 (5.3)	20.9 (5.2)	–
Category fluency (Z score)	−0.03 (0.98)	0.11 (1.06)	P = 0.44
HVLT-R delayed score	6.6 (2.9)	8.6 (2.0)	–
HVLT-R delayed (Z score)	−0.09 (1.05)	0.31 (0.74)	P = 0.010
RCFT delayed score	15.9 (8.1)	20.8 (5.4)	–
RCFT delayed (Z score)	−0.07 (1.05)	0.26 (0.77)	P = 0.034

Abbreviations: CN, Cognitively Normal; HC, Healthy Controls; HVLT, Hopkins Verbal Learning Test-Revised; JLO, Judgment of Line Orientation; LEDD, Levodopa Equivalent Daily Dose; MCI, Mild Cognitive Impairment; MDS-UPDRS, Movement Disorder Society-Unified Parkinson's Disease Rating Scale; MoCA-J, Japanese version of the Montreal Cognitive Assessment; PD, Parkinson's disease; RCFT, Ray–Osterrieth Complex Figure Test; SDMT, Symbol Digit Modalities Test; TMT, Trail Making Test; SD, Standard Deviation.

[a] Statistical tests were conducted using two-sample t-tests. Note that when comparing the results of the neuropsychological tests, the scores obtained from both HCs and patients with PD were converted to Z scores adjusted for age, sex, and education years using a linear regression model.

[b] These items had missing data for ten participants.

[c] Two participants had missing data for these items.

[d] The TMT is a test where longer completion times indicate abnormality. Higher TMT scores/Z-scores reflect these longer completion times, indicating poorer performance.

uptake ratio were differentially associated with subdomains of cognitive functions. After adjusting for age, sex, TBV, and the caudate uptake ratio, Ch4 volumes were correlated with verbal memory (HVLT-R delayed scores; r = 0.28, 95 % CI = 0.11 to 0.42, P = 0.0011) (Fig. 2b and Supplementary Table S6). In contrast, after adjusting for age, sex, and Ch4 volumes, the caudate uptake ratio was correlated with attention (SDMT scores, TMT-A time), executive function (TMT-B time), visuospatial domains (JLO scores), and visual memory (RCFT delayed scores) (Fig. 2b and Supplementary Table S6). Regarding the correlation between other BF regions (i.e., Ch1-2 and Ch3) and cognitive function, no correlations were observed (Supplementary Fig. S1c and Supplementary Table S7).

3.4. Mediation analysis within Ch4 volumes, cognitive data, and each region correlated with Ch4 volumes

The basal forebrain cholinergic system plays a modulatory role in wide range of cortical functions [21]. As we found correlations between Ch4 volumes and HVLT-R delayed scores, and between Ch4 volumes and GM volume loss in the medial temporal lobe, we performed causal mediation analysis between Ch4 volume and HVLT-R delayed scores, setting each regional GM volume in the amygdala, entorhinal cortex, hippocampus, fusiform gyrus, middle and inferior temporal gyri, and middle and posterior cingulate gyri as the mediator separately. When conducting this causal mediation analysis, we employed Bonferroni correction by dividing the significance level (α = 0.05) by the number of statistical tests conducted (n = 8) to tackle the challenge of multiple comparisons. We established an adjusted significance threshold of P < 0.00625 (0.05/8) to determine statistical significance. We found that only hippocampus volumes had significant mediation effects for Ch4 volumes on HVLT-R delayed scores (Fig. 3a). The mediation analysis revealed that hippocampus-mediated (i.e., ACME) effects of Ch4 volumes on HVLT-R delayed scores were significant (estimates = 0.013, 95 % CI = 0.006 to 0.020, P < 0.001), although direct (i.e., ADE) effects of Ch4 volumes on HVLT-R delayed scores were not significant (estimates = 0.008, 95 % CI = −0.005 to 0.020, P = 0.23) (Fig. 3b). The hippocampus-mediated proportion of the total (indirect + direct) effects of Ch4 volumes on HVLT-R delayed scores was 0.60. Fig. 3c displays path diagrams of the mediation analysis. Additionally, we conducted complementary mediation analysis between hippocampus volumes and HVLT-R delayed scores setting Ch4 volumes as the mediator. This mediation analysis revealed that the ACME of hippocampus volumes on HVLT-R delayed scores was not significant (estimates = 0.001, 95 % CI = −0.001 to 0.001, P = 0.19), although the ADE of hippocampus volumes on HVLT-R delayed was significant (estimates = 0.004, 95 % CI = 0.002 to 0.005, P < 0.001). Additional analyses between each cognitive function and GM volume loss aligned with the result indicating that the hippocampus mediates the correlation between Ch4 volume and HVLT-R delayed scores (Supplementary Fig. S2 and Supplementary Table S8).

3.5. Partial correlation between Ch4 volumes and cognitive data adding hippocampus volumes

The mediation effects of hippocampus volumes were significant for the relationship between Ch4 volumes and HVLT-R delayed scores. Therefore, to reinforce this result, we also performed Pearson's partial correlations between Ch4 volumes and HVLT-R delayed scores, adding hippocampus volumes as one of the covariates. The results showed that the correlation between Ch4 volumes and HVLT-R delayed scores became weak, with statistical significance being lost (r = 0.11, P = 0.19), when hippocampus volumes were additionally incorporated into the covariates.

3.6. Subgroup analysis

We divided 65 patients into the PD-CN group and 72 patients into the PD-MCI/PDD group. Clinical and imaging characteristics of the two groups were provided in Supplementary Table S9 and Supplementary Fig. S3. In both PD-CN and PD-MCI/PDD groups, the VBM results showed correlations of Ch4 volumes with regions similar to the primary results, including the medial temporal lobe, although more localized compared to the primary results. However, regarding the partial correlation and mediation analyses, only the PD-MCI/PDD group showed similar results to the overall cohort (i.e., Ch4 volume correlated with verbal memory and the hippocampus mediated these correlations), whereas in the PD-CN group, Ch4 volume did not show significant correlations with the cognitive function tests (Supplementary Fig. S4).

4. Discussion

In the "advanced-stage enriched" PD cohort, we explored the regions correlated with Ch4 volumes using VBM and the cognitive domains that were correlated with Ch4 volumes. The multiple linear regression model of VBM showed that Ch4 volume reduction was correlated with GM volume loss in the medial temporal lobe, including the hippocampus. Ch4 volumes were significantly correlated with verbal memory, whereas the caudate uptake ratio was correlated with attention, executive,

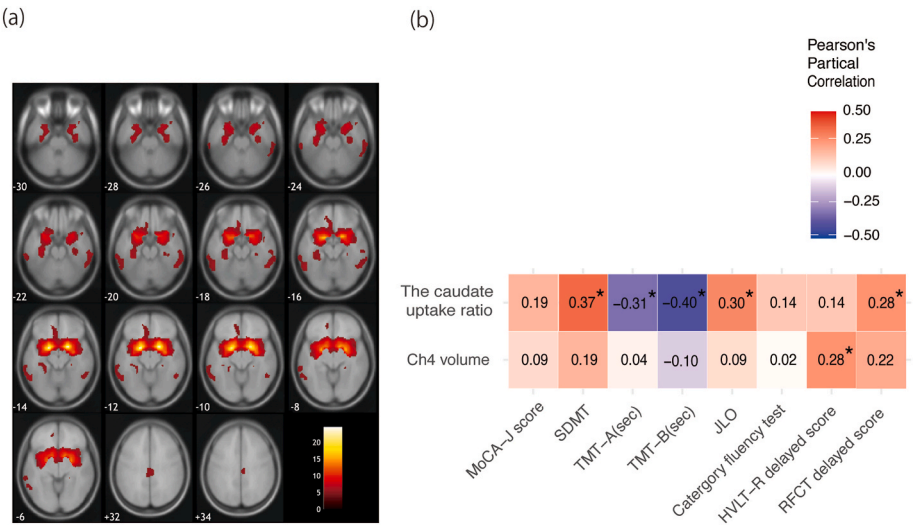

Fig. 2. Statistical parametric mapping T maps of brain areas of regional gray matter volume loss correlated with Ch4 volume reduction and heatmap of partial correlation coefficients between imaging parameters (Ch4 volume and caudate uptake ratio) and cognitive performance. (a) Statistical threshold is set at $P < 0.05$, family-wise error corrected, with clusters exceeding a spatial extent threshold of 29 voxels, which corresponds to the expected number of voxels per cluster. The color bar represents t scores. (b) For the analysis of Ch4 volumes, we adjusted for age, sex, total brain volume, and caudate uptake ratio, whereas for the analysis of caudate uptake ratio, we adjusted for age, sex, and Ch4 volumes. Asterisks represent significant effect threshold at $P < 0.0031$ (Bonferroni-corrected). HVLT-R, Hopkins Verbal Learning Test-Revised; JLO, Judgment of Line Orientation; MoCA-J, Japanese version of the Montreal Cognitive Assessment; RCFT, Ray–Osterrieth Complex Figure Test; SDMT, Symbol Digit Modalities Test; TMT, Trail Making Test.

visuospatial domains, and visual memory. Mediation analysis showed that hippocampus volume mediated the effects of Ch4 volume on verbal memory, suggesting that concomitant medial temporal lobe atrophy might play an intermediary role in the association between Ch4 atrophy and verbal memory impairment. Finally, our subgroup analyses showed that the hippocampus's intermediary role in the association between Ch4 atrophy and verbal memory impairment was evident in patients within the PD-MCI/PDD group.

We acknowledge that our findings, which show a specific correlation between Ch4 volume reduction and medial temporal lobe atrophy, but not between Ch1-2 volume reduction and medial temporal lobe atrophy, contrast with previous knowledge to some degree. For example, a previous study conducted in a very early-stage PD cohort indicated that the correlation with hippocampal volume was stronger for Ch1-2 volumes than for Ch4 volumes [22]. Additionally, we also acknowledge that there is a direct connection between Ch1-2 and the hippocampus, but not between Ch4 and the hippocampus [5]. However, we believe that this discrepancy can be explained by the differential involvement of α-synuclein pathology in Ch1-2 and Ch4 during the disease course. Specifically, considering α-synuclein pathology [12], in Braak stage 3, the magnocellular nuclei of the BF and the second sector of Ammon's horn are involved. In Braak stage 4, α-synuclein pathology extends to the accessory cortical and central nuclei of the amygdala and anteromedial temporal mesocortex. In Braak stage 5, the extension to the neocortex is centered on the temporal mesocortex. Therefore, α-synuclein pathology appears in the hippocampus and Ch4 at the same stage of the disease, and as the disease progresses to more advanced stages, the α-synuclein pathology in both regions becomes similarly severe [23]. Notably, in contrast to the consistent α-synuclein pathology involvement of Ch4 in PD, the involvement of Ch1-2 is variable [23,24]. Therefore, we speculate that in the early stage of PD, when the damage from α-synuclein pathology is not so severe, Ch1-2 and hippocampal volume are correlated, possibly reflecting a direct interaction. In the advanced stage, when the damage from α-synuclein pathology becomes severe and PD-MCI/PDD develops, the effect of α-synuclein pathology predominates, strengthening the correlation between Ch4 and hippocampal volume while weakening the correlation between Ch1-2 and hippocampal volume. Indeed, in our cohort, partial correlation analyses between Ch1-2 and hippocampal volume after controlling for age, sex, and TBV showed that Ch1-2 volume significantly correlated with hippocampus volume in the PD-CN groups ($r = 0.25$, 95 % CI = 0.003 to 0.46, $P = 0.048$) but not in the PD-MCI/PDD group ($r = 0.06$, 95 % CI = −0.18 to 0.28, $P = 0.65$), supporting the abovementioned theory.

As highlighted in a previous study [8], patients with early-stage non-demented PD showed a correlation between BF atrophy and cortical cholinergic deficits, both of which are associated cognitive impairment in this phase. However, another previous study of de novo PD patients indicated that BF atrophy alone did not drive global apparent cognition differences in PD [4], suggesting that additional factors may additionally contribute to more severe cognitive impairment. In light of our finding that the hippocampus plays an intermediary role in the association between Ch4 atrophy and verbal memory impairment in an "advanced-stage enriched" PD cohort, as well as subgroup analyses showing that these mediations are evident in the PD-MCI/PDD group, we propose that the hippocampal atrophy is the primary aforementioned "other additional factor." This proposal should be plausible considering previous reports showing more atrophy in the amygdala, entorhinal cortex, and hippocampus in PD-MCI/PDD than in HCs and PD-CN; these changes were associated with verbal memory impairment [25,26]. Furthermore, although it is discussed in the context of AD, it was shown that apparent cognitive dysfunction first manifests when medial temporal lobe atrophy is added to Ch4 atrophy [10]. However, how does this proposal explain our mediation analysis result (i.e., the impact of Ch4 atrophy on verbal memory function was primarily mediated by hippocampal atrophy)? We believe that the clue lies in the effect of Ch4 atrophy on the memory function of the hippocampus. Within the cortex, acetylcholine enhances the response to sensory input, which plays a critical role in processes related to memory formation, including those involving the hippocampus [21]. More importantly, a recent study in humans where deep brain stimulation was applied to the NBM, it has been suggested that there exists "functional" connectivity between the NBM and medial temporal lobe structures, including the hippocampus [27]. Therefore, we believe that our mediation analysis result can be well explained if we consider that in the cognitively-impaired stage of PD, the volume loss of Ch4 (a well-known cholinergic "hub" in the brain) contributes to cognitive dysfunction

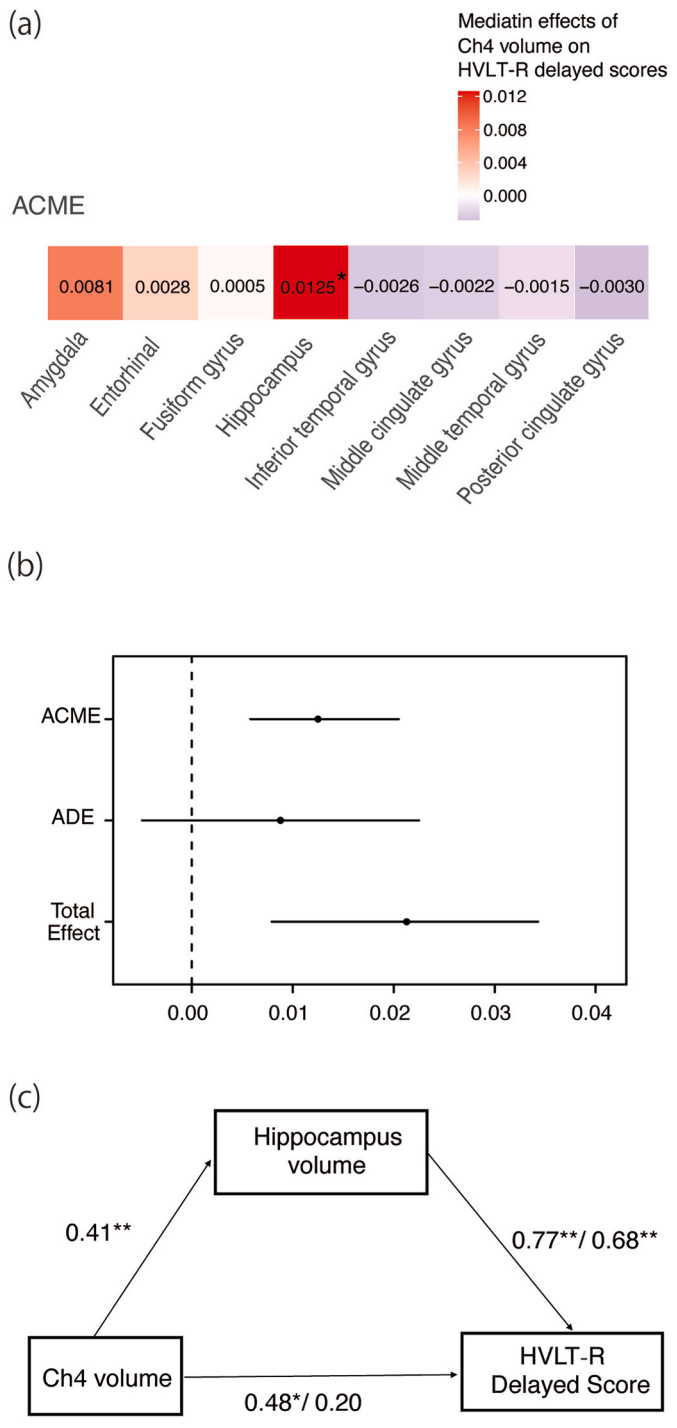

Fig. 3. Heatmap of mediation effects of Ch4-related regional gray matter reduction for Ch4 volume loss on Hopkins Verbal Learning Test-Revised (HVLT-R) delayed scores. (a) Asterisks represent significant effects as per mediation analyses ($P < 0.001$). (b) The estimated mediation effects of hippocampus volumes for Ch4 volume loss on HVLT-R delayed scores. The total effects describe the sum of indirect and direct effects of Ch4 volumes on HVLT-R delayed scores. (c) Path diagrams of the mediation analysis. Diagrams include standardized regression coefficients for each path in the model. Coefficients after slashes show path values adjusted for the mediation effect. Coefficients * and ** correspond to significant associations ($P < 0.05$ and $P < 0.001$). ACME, Average Causal Mediation Effect; ADE, Average Direct Effect; HVLT-R, Hopkins Verbal Learning Test-Revised.

mainly through exacerbating hippocampal memory function. Although this proposal warrants future validation studies, we nonetheless believe that synergistic disturbances between Ch4 and the hippocampus are crucial in exacerbating severe cognitive deterioration in PD-MCI/PDD.

Additionally, our study revealed that the caudate uptake ratio was correlated with attention, executive, visuospatial domains, and visual memory. A previous study showed that the dopaminergic function of the caudate was correlated with attention and executive domains as part of the frontostriatal system [28]. Studies also revealed that the dopaminergic function of the caudate was correlated with the visuospatial and visuospatial memory domains, similar to our findings [29,30], although this correlation was not consistent. The physiological mechanism underlying the correlation between dopaminergic function and visuospatial and visuospatial memory domains remains unclear.

Several limitations of this study should be acknowledged. First, our study lacked CSF data such as Aβ, phosphorylated tau, and total tau levels. Second, because our study was cross-sectional, it is unclear whether Ch4 volume loss preceded the medial temporal lobe atrophy. Longitudinal data would provide a more accurate means of estimating causal relationships in causal analysis. Third, in addition to caudate dopaminergic dysfunction evaluated in the present study, frontal dopaminergic disturbance may also contribute to frontostriatal deficits in patients with PD, which could not be determined.

In conclusion, we demonstrated that in this advanced-stage enriched PD cohort, Ch4 volume is correlated with verbal memory function and, as shown by VBM, is associated with medial temporal lobe volume. Our results suggest that Ch4 volume atrophy causes verbal memory dysfunction potentially via concomitant medial temporal lobe atrophy in the advanced-stage PD, possibly reflecting concomitant α-synuclein pathology in these regions.

Data statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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CRediT authorship contribution statement

Haruhi Sakamaki-Tsukita: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Atsushi Shima:** Writing – review & editing, Supervision, Software, Methodology, Data curation. **Daisuke Kambe:** Data curation. **Koji Furukawa:** Data curation. **Akira Nishida:** Data curation. **Ikko Wada:** Data curation. **Kenji Yoshimura:** Data curation. **Yusuke Sakato:** Data curation. **Yuta Terada:** Data curation. **Hodaka Yamakado:** Data curation. **Yosuke Taruno:** Data curation. **Etsuro Nakanishi:** Data curation. **Masanori Sawamura:** Data curation. **Yasutaka Fushimi:** Data curation. **Tomohisa Okada:** Data curation. **Yuji Nakamoto:** Supervision, Data curation. **Laszlo Zaborszky:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Ryosuke Takahashi:** Writing – review & editing, Supervision. **Nobukatsu Sawamoto:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Ryosuke Takahashi received a research grant from Nihon Medi-Physics Co., Ltd., a manufacturer of ^{123}Iioflupane.

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Appendix A. Supplementary data

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