Differential synaptic loss in β-amyloid positive versus β-amyloid negative corticobasal syndrome

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Abstract

Background/Objective: The corticobasal syndrome is a complex asymmetric movement disorder, with cognitive impairment. Although commonly associated with the primary 4R-tauopathy of corticobasal degeneration, clinicopathological correlation is poor, and a significant proportion are due to Alzheimer’s disease (AD). Synaptic loss is a pathological feature of many clinical and preclinical tauopathies. We therefore measured the degree of synaptic loss in patients with corticobasal syndrome and tested whether synaptic loss differed according to β-amyloid status.

Methods: Twenty-five people with corticobasal syndrome (CBS), and thirty-two age-/sex-/education-matched healthy controls participated. Regional synaptic density was estimated by \([^{11}C]\text{UCB-J}\) non-displaceable binding potential (BP\(_{ND}\)), AD-tau pathology by \([^{18}F]\text{AV-1451}\) BP\(_{ND}\), and grey matter volume by T1-weighted MRI. Participants with CBS had β-amyloid imaging with \([^{11}C]\text{PiB}\) PET. Symptom severity was assessed with the PSP-rating-scale, the cortical basal ganglia functional scale, and the revised Addenbrooke’s Cognitive Examination. Regional differences in BP\(_{ND}\) and grey matter volume between groups were assessed by ANOVA.

Results: Compared to controls, patients with corticobasal syndrome had higher \([^{18}F]\text{AV-1451}\) uptake, grey matter volume loss and reduced synaptic density. Synaptic loss was more severe and widespread in the β-amyloid negative group. Asymmetry of synaptic loss was in line with the clinically most affected side.

Discussion: Distinct patterns of \([^{11}C]\text{UCB-J}\) and \([^{18}F]\text{AV-1451}\) binding and grey matter volume loss, indicate differences in the pathogenic mechanisms of the corticobasal syndrome according to whether it is associated with the presence of Alzheimer’s disease or not. This highlights the potential for different therapeutic strategies in corticobasal syndromes.
**Introduction**

The corticobasal syndrome (CBS) is a highly heterogeneous neurodegenerative disorder. It is characterised by the combination of a movement disorder (akinetic-rigidity, dystonia, myoclonus, alien-limb or apraxia) with cognitive decline (affecting language, visuospatial, executive function and memory domains). At post mortem, CBS is often associated with the 4-repeat tauopathy of corticobasal degeneration (CBD), with some cases of the closely related tauopathy of progressive supranuclear palsy (PSP). However, the pathological correlation with clinical diagnostic criteria is poor, and pathologies other than CBD and PSP account for 30-50% of cases; the most frequent alternative pathology being Alzheimer’s disease (AD). Such pathophysiological heterogeneity presents not only a clinical diagnostic challenge, but also an obstacle to rational mechanisms-based therapeutic strategies and clinical trials design.

Here we focus on the difference between AD and non-AD causes of corticobasal syndrome, where the non-AD causes are most likely to be CBD (or PSP). They differ in their associated molecular pathologies (4-repeat tau in CBD/PSP, versus 3-/4-repeat tau with β-amyloid pathology in AD), and biomarkers in blood, cerebrospinal fluid, and neuroimaging. Fluorodeoxyglucose PET imaging differs between β-amyloid-positive and β-amyloid-negative patients. MRI shows asymmetric cortical and subcortical grey and white matter changes, but the ability of structural MRI to differentiate the molecular pathology of CBS is limited.

Synaptic loss is common in preclinical models and neuropathological studies of diverse neurodegenerative diseases, including multiple tauopathies. It is a convergence point for the neurotoxicity of misfolded protein aggregation, mitochondrial stress and neuroinflammation; and occurs before neurodegeneration in transgenic tauopathies. The radioligand \[^{11}C\]UCB-J can be used to estimate synaptic density in vivo, based on its affinity for the presynaptic vesicle glycoprotein SV2A. This ligand reveals significant synaptic loss in Alzheimer’s disease, frontotemporal dementia, Lewy body dementia, Parkinson’s disease, and progressive supranuclear palsy. The synaptic loss correlates with clinical disease severity and is more closely correlated with severity than the level of β-amyloid, tau or atrophy, in Alzheimer’s disease, progressive supranuclear palsy, and frontotemporal dementia.

The aim of this study was to examine the heterogeneity of corticobasal syndrome in terms of synaptic density, as measured by \[^{11}C\]UCB-J PET, compared to \[^{18}F\]AV-1451 uptake and grey matter volume loss. Our principal hypotheses were that: a) the extent and severity of synaptic loss differs according to β-amyloid status; and b) the spatial distribution of synaptic loss...
correlates with the patients’ clinically most affected side. Secondary hypotheses were that c) 
\[^{18}F\]AV-1451 uptake and volume loss differ according to β-amyloid status.
Methods

Participants

Twenty-five people with possible or probable CBS, according to the Armstrong criteria, were recruited from regional specialist clinics at the Cambridge University Centre for Parkinson-plus, and National Hospital for Neurology and Neurosurgery, London. Thirty-two healthy volunteers were recruited from the National Institute for Health Research Join Dementia Research register. Participants were screened using the inclusion/exclusion criteria set out in Holland et al. 2020. All participants undertook synaptic imaging with \([^{11}C]UCB-J\) PET, and tau imaging with \([^{18}F]AV-1451\) (flortaucipir) PET. Participants with CBS had amyloid PET imaging using Pittsburgh Compound B \(([^{11}C]PiB)\).

Participants undertook the revised Addenbrooke’s Cognitive Examination (ACE-R) and mini-mental state examination; disease severity was measured with the PSP rating scale (aimed at diseases caused by 4-repeat tauopathies), and the Cortical Basal ganglia Functional Scale (CBFS). The most clinically affected body side (as per patient reported symptoms or clinician review) was recorded. Functional disease severity was rated by the Cambridge Behavioural Inventory (CBI), and the Clinical Dementia Rating Scale sum-of-boxes (CDR).

Standard Protocol Approvals, Registrations, and Patient Consents

The study was approved by the Cambridge Research Ethics Committee (18/EE/0059) and the UK Administration of Radioactive Substances Advisory Committee. Participants provided written informed consent in accordance with the Declaration of Helsinki.

Neuroimaging

\([^{11}C]UCB-J\) PET and grey matter volume (MRI)

The procedure for \([^{11}C]UCB-J\) synthesis, dynamic PET data acquisition, image reconstruction and kinetic analysis are reported in detail in Holland et al 2020, 2022. In brief, dynamic PET data acquisition was performed on a GE SIGNA PET/MR (GE Healthcare, Waukesha, USA) for 90 minutes immediately after injection, with attenuation correction using a multi-subject atlas method. Emission images were aligned using SPM12 (www.fil.ion.ucl.ac.uk/spm/software/spm12/), and rigidly registered to a T1-weighted MRI
acquired during PET data acquisition (TR = 3.6 msec, TE = 9.2 msec, 192 sagittal slices, in plane resolution 0.55 x 0.55 mm, interpolated to 1.0 x 1.0 mm; slice thickness 1.0 mm). For regional analysis, we used a modified version of the n30r83 Hammersmith atlas (http://brain-development.org) including segmentation of brainstem and cerebellar structures, with the atlas non-rigidly registered to the T1-weighted MRI of each participant, using the Advanced Normalisation Tools (ANTS) software. Grey matter volumes were extracted using SPM12 segmentation, for all 95 Hammersmith atlas regions, with 78 brain regions (excluding the ventricles and the corpus callosum) taken forward to regional analysis. Detailed results for all 78 regions are given in Supplementary Tables 1 and 2, and Figure 2 and Supplementary Figure 2. Aggregate regional results (frontal lobe, parietal lobe, temporal lobe, occipital lobe, and central structures) are presented in the main manuscript tables for ease of presentation (Table 2).

Correction for partial volume error from cerebrospinal fluid was applied to each dynamic PET image, and regional time-activity curves extracted. This corrects for the potential presence of CSF in some of the image volumes, which a priori has no synapses. To assess the impact of partial volume correction, time-activity curves were also extracted from the same regions of interest without the application of partial volume correction (discussed in the supplementary material as “without partial volume correction”).

To quantify synaptic density, \[[11C]UCB-J non-displaceable binding potential (BP_{ND}) was determined using a basis function implementation of the simplified reference tissue model, with the reference tissue defined in the centrum semiovale.\]

\[
[18F]AV-1451 PET
\]

Dynamic [18F]AV-1451 PET imaging was performed on a GE SIGNA PET/MR for 90 minutes after [18F]AV-1451 injection. [18F]AV-1451 BP_{ND} was determined using a basis function implementation of the simplified reference tissue model, with the reference tissue defined in the inferior cerebellar grey matter, using a 90% threshold on the grey matter probability map, produced by SPM12 smoothed to PET resolution. We acknowledge that [18F]AV-1451 has high affinity for AD-related tau, and low affinity for the 4R-tau of CBD.

\[
[11C]PiB PET
\]

B-amyloid imaging using Pittsburgh Compound B ([11C]PiB) followed Holland et al 2020. [11C]PiB cortical standardised uptake value ratio (SUVR; 50-70 minutes post injection) was calculated using the whole cerebellum reference tissue as per the Centiloid Project
methodology. A negative amyloid status was characterised by a cortical $[^{11}\text{C}]$PiB SUVR less than 1.21 obtained by converting the Centiloid cut-off of 19 to SUVR using the Centiloid-to-SUVR transformation in Jack et al 2017. Patients with an SUVR > 1.21 are referred to as ‘CBS/Aβ+ve’ and those with SUVR < 1.21, ‘CBS/Aβ-ve’.

All $[^{11}\text{C}]$UCB-J PET and T1 MRI occurred contemporaneously. $[^{18}\text{F}]$AV-1451 and $[^{11}\text{C}]$PiB PET imaging occurred close to $[^{11}\text{C}]$UCB-J PET (AV-1451: Median -3 months; PiB: Median +2 months).

**Statistical analyses**

All statistical analyses were implemented in R (version 4.2.0). We compared demographic and clinical variables between patients and controls, using ANOVA, or chi-square tests as appropriate.

Linear regression models were used to test for the effect of $[^{11}\text{C}]$UCB-J and $[^{18}\text{F}]$AV-1451 binding, and grey matter volume on disease severity rating scales (PSPRS, CBFS, and ACE-R), allowing for an interaction with brain region, with age as a covariate.

**Assessing asymmetry in $[^{11}\text{C}]$UCB-J and $[^{18}\text{F}]$AV-1451 BPND and grey matter volume:**

The laterality index for synaptic density, from regional $[^{11}\text{C}]$UCB-J BPND values, was calculated as follows, and correlated with laterality of clinical symptoms:

\[
\text{Laterality Index (LI)} = \frac{(\text{Left UCB-J BPND} - \text{Right UCB-J BPND})}{(\text{Left UCB-J BPND} + \text{Right UCB-J BPND})}
\]

The laterality index ranges from -1 to +1, indicating left and right dominant synaptic loss on $[^{11}\text{C}]$UCB-J PET respectively, with 0 indicating symmetry (Figure 1). We performed a two-way paired-sample ANOVA to test for a difference in $[^{11}\text{C}]$UCB-J BPND in left versus right brain regions (using 11 aggregate brain regions – frontal, temporal, parietal, occipital lobes, cingulate cortex, and central structures). A post-hoc analysis tested for region-by-side interactions (Bonferroni corrected for multiple comparisons, alpha = 0.0045). A binomial logistic regression tested the relationship between the most affected side on clinical examination versus that on $[^{11}\text{C}]$UCB-J PET.

The laterality index for $[^{18}\text{F}]$AV-1451 uptake and grey matter volume followed the same steps as for $[^{11}\text{C}]$UCB-J above.
Differences in $[^{18}F]AV-145$ binding, $[^{11}C]UCB-J$ binding, and grey matter volume between patients and controls, and within patients based on $\beta$-amyloid status:

We compared regional $[^{11}C]UCB-J$ and $[^{18}F]AV-145$ BPND, and grey matter volume between patients and controls, and within the patient cohort (CBS/A$\beta$+ve versus CBS/A$\beta$-ve); we used ANCOVA with age as covariate (and total intracranial volume in the case of grey matter volume comparison). Statistical inferences were corrected for multiple comparisons (Tukey’s HSD method), and the resultant t-statistics projected onto brain maps with the following contrasts: control-more-than CBS/A$\beta$+ve, control-more-than CBS/A$\beta$-ve, CBS/A$\beta$+ve more-than CBS/A$\beta$-ve. Regional $[^{11}C]UCB-J$ and $[^{18}F]AV-145$ BPND, and grey matter volumes were standardised against the control data; the resulting z-scores were used to calculate effect sizes (Cohen’s d) for group comparisons.

Given the small numbers of people with CBS in our study, we corroborated our frequentist statistics, with a Bayesian analysis using the software JASP. For each of the abovementioned group comparisons, we report the Bayes factor (BF10) for the alternative hypothesis (that there is a group difference) over the null hypothesis (that there is no group difference) with the following BF interpretations: $>100 =$ extreme, $>30 =$ very strong, $>10 =$ strong, $>3 =$ moderate, and 1-3= anecdotal evidence for the alternative hypothesis; 0.33–1= anecdotal, $<0.33=$ moderate, $<0.10 =$ strong, $<0.03 =$ very strong, $<0.01 =$ extreme evidence for the null hypothesis (Supplementary Table 3A-B).

Data availability

Anonymised derived data at subject- region- and modality-level that support the findings of this study are available from the corresponding author without restriction. Clinical and raw imaging data may be requested, but are subject to restrictions and likely need for a material transfer agreement to preserve participant confidentiality.
Results

Demographics and clinical characteristics

Patients and controls were matched in age, sex, and education. Impairments were seen across multiple cognitive domains of the ACE-R and MMSE. There were high endorsements on the CBI, and high scores on the CDR sum-of-boxes. Seventeen out of 25 participants with CBS were β-amyloid-negative (CBS/Aβ-ve: SUVR < 1.21), and eight positive (CBS/Aβ+ve).

The cognitive screening test performance, symptom severity and duration, and carer burden questionnaires were similar between CBS/Aβ-ve and CBS/Aβ+ve subgroups. Specifically, there were no differences in symptom duration or severity on the PSP/CBFS rating scales. Similar scores were seen on tests of cognition, and carer endorsements (Table 1; p>0.05, and BF10 < 1 in support of the null hypothesis). The clinically most affected side was on the right for ten patients, and on the left for 14 patients. One patient had presented with asymmetric symptoms but was clinically symmetrical by the time of PET imaging and was excluded from the asymmetry analysis.

*** Table 1 ***

Relationship between imaging parameters and clinical severity scales

Across all patients, higher scores on the PSP rating scale were associated with lower \[^{11}C\]UCB-J binding (β= -3.0, F(p) = 14.1 (<0.01)), and higher \[^{18}F\]AV-1451 binding (β= 1.1, F(p) = 12.8 (0.03)), but not associated with grey matter volume loss or age. Similarly, higher scores on the Cortical Basal ganglia Functional Scale (CBFS), were associated with lower \[^{11}C\]UCB-J binding (β= -18.7, F(p) = 8.7 (<0.01)), particularly within the bilateral parietal lobes, bilateral middle and inferior frontal gyri, right precentral gyrus and right posterior temporal lobe. Higher CBFS scores were associated with higher \[^{18}F\]AV-1451 binding (B = 27.9, F(p) = 98.9 (<0.001)), and age ((β= 0.15, F(p) = 7.8 (<0.01)). Better performance on the ACE-Revised was associated with higher \[^{11}C\]UCB-J binding (β= 6.7 F(p) = 51.1 (<0.001)), higher grey matter volume (β= 0.1 F(p) = 64.4 (<0.001)), younger age (β= -0.12, F(p) = 36.0 (<0.001)), and lower \[^{18}F\]AV-1451 binding (β= -36.7, F(p) = 160.3 (<0.001)).
Asymmetric $^{[11]}$C$\text{UCB-J}$ and $^{[18]}$F$\text{AV-1451}$ binding and grey matter atrophy

Across all CBS patients, there was asymmetric $^{[11]}$C$\text{UCB-J}$ and $^{[18]}$F$\text{AV-1451}$ binding and grey matter atrophy. $^{[11]}$C$\text{UCB-J}$ BPND was asymmetric within the following brain regions: frontal and occipital lobes ($p=0.01$), cingulate ($p<0.0001$), insula ($p<0.0001$), thalamus ($p=0.01$), caudate nucleus ($p<0.0001$), pallidum ($p<0.0001$), and the putamen ($p=0.03$), with the pallidum, caudate nucleus, cingulate, and insula significant after correction for multiple comparisons. The most affected side on $^{[11]}$C$\text{UCB-J}$ imaging, correlated with clinical severity in the contralateral body part (logistic regression: $\beta = -1.4$, $p<0.0001$), and in the case of asymmetry in the cerebellum only, with the ipsilateral body part (logistic regression: $\beta = 3.4$, $p<0.006$) (Figure 1, and supplementary Figure 1).

$^{[18]}$F$\text{AV-1451}$ BPND was asymmetric within the frontal ($p = 0.03$) and the parietal lobe ($p = 0.04$), uncorrected. The side affected most on imaging was not associated with clinical severity in the contralateral body part. Grey matter atrophy (adjusted for total intracranial volume) was asymmetric within the following brain regions: frontal lobe ($P<0.001$), temporal ($P<0.001$), parietal lobe ($P<0.01$), cingulate ($P<0.001$), insula ($P<0.001$), putamen ($P<0.01$), and thalamus ($P<0.01$), with all but the parietal lobe and the putamen significant after correction for multiple comparison. Asymmetry on atrophy was not significantly associated with asymmetry on clinical assessment.

***Figure 1***
Differences in $[^{18}\text{F}]$AV-1451 and $[^{11}\text{C}]$UCB-J binding, and grey matter volume between patients and controls, and within patients based on $\beta$-amyloid status

Table 2 (aggregate regions; partial volume corrected), and detailed Supplementary Tables 1A-C (partial volume corrected) and 2A-C (without partial volume correction) summarise the t values, Cohen’s d effect sizes and p-values for the regional comparisons in $[^{18}\text{F}]$AV-1451 and $[^{11}\text{C}]$UCB-J BPND, and grey matter volume, between patients and controls, and within patients. For detailed visualisation, the t values from the 78 brains regions in Supplementary Tables 1A-C and 2A-C (thresholded at adjusted p <0.05), are illustrated on brain maps in Figure 2A (partial volume corrected), and Supplementary Figure 2A (partial volume uncorrected). In both figures, higher t-values depict higher $[^{18}\text{F}]$AV-1451 binding, higher $[^{11}\text{C}]$UCB-J binding, and higher grey matter volume.

**Controls-more-than CBS/A$\beta$+ve**

*Frequentist approach:*

Compared to controls, the CBS/A$\beta$+ve group had increased $[^{18}\text{F}]$AV-1451 binding in 51 (out of 78) frontal, temporal, parietal and occipital subregions, in addition to the cingulate, insula and the left putamen (t score range: -2.59 to -4.68; Cohen’s d range: -0.89 to -1.60, p<0.05).

Conversely, $[^{11}\text{C}]$UCB-J binding was reduced only in 12 subregions including the right middle frontal gyrus, the pre- and postcentral gyri, bilateral superior parietal gyri, posterior temporal and lateral occipital lobes with large effect sizes (t value range: 2.43 to 4.93, Cohen’s d range: 0.83 to 2.15, p<0.05). Widespread cortical and subcortical grey matter volume loss was observed in 46 subregions of the frontal, parietal, temporal and occipital lobes, as well as the caudate and putamen and thalamus with at least moderate to large affect sizes (t value range: 2.47 to 6.49, Cohen’s d range: 0.76 to 2.45, p<0.05) (Table 2A, Supplementary Table 1A, Figure 2A – left-hand column).

*Bayesian approach:*

Bayesian statistics corroborate with the results obtained through the frequentist approach above. For differences in $[^{18}\text{F}]$AV-1451 binding between CBS/A$\beta$+ve and controls, BF$_{10}$ were >3 in fifty regions of interest including fronto-temporal-parietal regions, and putamen. For differences in $[^{11}\text{C}]$UCB-J BP$_{ND}$ between CBS/A$\beta$+ve and controls, BF$_{10}$ was > 3 in forty-four regions, and >100 in temporal-parietal-occipital regions, cingulate, and subregions of the frontal lobe (precentral gyri, and middle frontal gyrus). There was widespread grey matter
volume loss, with moderate evidence ($BF_{10} > 3$) in 62 regions in CBS/Aβ+ve (Supplementary Table 3A).

**Controls-more-than CBS/Aβ-ve**

**Frequentist approach:**
Compared to controls, the CBS/Aβ-ve group had increased [$^{18}$F]AV-1451 binding in 3 subregions: the left pallidum, right substantia nigra and the midbrain (t value range: -2.45 to -2.82; Cohen’s d range: -0.71 to -0.84, p<0.05). [$^{11}$C]UCB-J binding was reduced in nearly all subregions (72/78) across both the cortical mantle and subcortical areas (t value range: 2.46 to 5.69, Cohen’s d range: 0.74 to 2.09, p<0.05). Cortical and subcortical grey matter volume loss was observed in 32 subregions including the anterior orbital gyri, middle frontal gyrus, parietal lobe subregions, posterior temporal lobe, caudate nucleus, putamen, thalamus and the cerebellum (t value range: 2.42 to 5.01, Cohen’s d range: 0.82 to 1.76, p<0.05) (Table 2B, Supplementary Table 1B, and Figure 2A – middle column).

**Bayesian approach:**
$BF_{10}$ values were much lower when comparing [$^{18}$F]AV-1451 binding in CBS/Aβ-ve and controls, with values > 3 in the precentral gyri, pallidum, substantia nigra, midbrain and cerebellar grey matter. Echoing the frequentist results above, there was strong evidence ($BF_{10} > 10$) in support of lower [$^{11}$C]UCB-J binding in 78 regions with extreme evidence in 67 regions ($BF_{10} > 100$). There was moderate evidence ($BF_{10} > 3$) for widespread grey matter volume loss in 45 regions in the CBS/Aβ-ve, compared to controls (Supplementary Table 3A).

**CBS/Aβ+ve more-than CBS/Aβ-ve**

**Frequentist approach:**
As expected, compared to the CBS/Aβ-ve cohort, the CBS/Aβ+ve group had higher [$^{18}$F]AV-1451 binding in 48 subregions including all major cortical areas, posterior cingulate, and the cerebellar dentate (t value range: 2.50 to 4.06, Cohen’s d range: 0.82 to 1.25, p<0.05). The CBS/Aβ+ve cohort had higher [$^{11}$C]UCB-J binding in 21 subregions including the orbitofrontal gyri, the precentral gyrus, medial part of the anterior temporal lobe, parahippocampal and hippocampal regions, substantia nigra, pons and cerebellum (t value range: 2.42 to 3.68, Cohen’s d range: 0.64 to 1.35, p<0.05). The differences in [$^{11}$C]UCB-J binding between CBS/Aβ+ve and CBS/Aβ-ve patients within the frontal lobe, are primarily driven by
orbitofrontal subregions (Supplementary Table 2C) – this may account for the lack of a
difference between these two groups when larger aggregate regions are used (e.g. Frontal lobe),
as illustrated in Table 2C. Grey matter volume loss was more pronounced in CBS/Aβ+ve
patients in the superior parietal gyrus, anterior temporal lobe, and caudate nucleus (t value
range: -2.49 to -3.26, Cohen’s d range: -0.92 to -1.56, p<0.05), with the CBS/Aβ-ve showing
a lower left substantia nigral volume (t=2.63, Cohen’s d 0.85, p=0.03) (Table 2C,
Supplementary Table 1C, and Figure 2A – right-hand column).

Bayesian approach:
For differences in [18F]AV-1451 uptake between CBS/Aβ+ve and CBS/Aβ-ve, there was
positive evidence for higher cortical binding in CBS/Aβ+ve (BF10 > 3), and moderate evidence
for no difference in brainstem uptake between the two patient cohorts (BF10 < 0.3). For
differences in [11C]UCB-J BPND, there was anecdotal evidence supporting higher binding in
CBS/Aβ+ve in 18 regions, and moderate evidence (BF10 > 3) in three regions: anterior and
lateral orbital gyri, and the cerebellar dentate). CBS/Aβ+ve patients had more significant
atrophy within the right caudate, medial orbital gyrus, right anterior cingulate, right lingual
gyrus, and right antero-lateral temporal lobe (BF10 > 3).

The distinct patterns of [18F]AV-1451 BPND uptake and synaptic loss ([11C]UCB-J BPND) in
CBS/Aβ+ve and CBS/Aβ-ve cohorts are readily appreciated in the scatterplot in Figure 2B,
where at a regional level, patients who are β-amyloid positive have higher [18F]AV-1451
binding and less synaptic loss compared to controls, than those who are β-amyloid negative. In
both cohorts, there was a negative relationship between [18F]AV-1451 uptake and [11C]UCB-J
BPND (CBS/Aβ+ve: R = -0.49 (p < 0.001); CBS/Aβ-ve: R = -0.36 (p = 0.001)).

Analyses using partial volume uncorrected data (Supplementary Tables 2A-C, Supplementary
Table 3B, and Supplementary Figure 2) show slightly more severe synaptic loss in both
CBS/Aβ+ve and CBS/Aβ-ve groups compared to controls. However, the distribution of
synaptic loss and the differential patterns of loss seen in CBS/Aβ+ve versus CBS/Aβ-ve cohorts
remains similar to corrected data, and overall, the results largely echo the findings above,
indicating that the differences in synaptic loss are not merely an artefact of atrophy or atrophy
correction.
**Table 2 (A-C)**

***Figure 2A & Figure 2B***
Discussion

There are three main findings of this study. First, the degree and distribution of synaptic loss is clinically correlated in people with CBS, not only in summary rating scales but also in accord with the laterality of clinical deficits. Second, there is a distinct pattern of synaptic loss, \[^{18}\text{F}]\text{AV-1451}\ binding, and grey matter volume loss, in people with CBS according to whether or not they have likely Alzheimer’s disease as the underlying pathology (i.e. are positive or negative for the \[^{11}\text{C}]\text{PiB}\ amyloid biomarker). Third, synaptic loss is more widespread and more severe in β-amyloid-negative patients with CBS.

Within our cohort, clinical signs were asymmetric in all but one case, in both CBS/Aβ+ve and CBS/Aβ-ve patients. This mirrored the asymmetry of synaptic loss, particularly within the contralateral caudate nucleus, thalamus, cingulate and ipsilateral cerebellum (Figure 1 and Supplementary Figure 1). Laterality on imaging and correlations with asymmetric clinical symptoms have previously been reported for grey matter atrophy, glucose metabolism (as indexed by \[^{18}\text{F}]\text{FDG PET}\), and \[^{18}\text{F}]\text{AV1451}\ binding, and now shown here in terms of asymmetric synaptic loss.

We found distinct patterns of \[^{18}\text{F}]\text{AV-1451}\ and \[^{11}\text{C}]\text{UCB-J}\ binding, and grey matter volume loss in CBS/Aβ+ve versus CBS/Aβ-ve patients, compared to controls. In CBS/Aβ+ve patients who are likely to have Alzheimer’s disease as the underlying pathology, \[^{18}\text{F}]\text{AV-1451}\ binding was diffuse in the cortical grey matter, with particularly high binding in the occipito-parietal lobes and the temporal lobe. Our findings echo reports from previous \[^{18}\text{F}]\text{AV-1451}\, and second generation tau PET tracers (e.g. \[^{18}\text{F}]\text{PI-2620}), with significant uptake mainly in posterior cortical areas. The extent and degree of grey matter volume loss in our CBS/Aβ+ve cohort, followed that of \[^{18}\text{F}]\text{AV-1451}\ binding but also included subcortical areas. \[^{11}\text{C}]\text{UCB-J}\ binding potential was mainly reduced in occipito-parietal regions (Supplementary Table 1A, and aggregate regions in Table 2A). The pattern of synaptic loss in our CBS/Aβ+ve cohort is similar to that reported in Alzheimer’s disease by Mecca et al. who also reported smaller effect sizes for synaptic loss than for grey matter volume loss.

The CBS/Aβ-ve patients are likely to have corticobasal degeneration as the underlying pathology, although we acknowledge that PSP or FTLD-tau are possible. We observed a minimal increase in \[^{18}\text{F}]\text{AV-1451}\ binding compared to controls, with only the brainstem, and basal ganglia withstanding correction for multiple comparisons. \[^{18}\text{F}]\text{AV-1451}\ has a high
binding affinity for the paired helical tau filaments in AD, with lower affinity for the straight filaments of 4-repeat tau found in CBD/PSP; its ability to detect non-AD dementias, and to differentiate between the primary tauopathies of CBD and PSP is limited.\textsuperscript{40, 50} In the CBS/Aβ-ve cohort, grey matter volume loss was extensive but far less severe than the extent and severity of cortical and subcortical synaptic loss, as shown by significantly reduced $[^{11}\text{C}]$UCB-J BP\textsubscript{ND} in nearly all subregions in Supplementary Table 1B (and aggregate regions in Table 2B), similar to findings in PSP.\textsuperscript{26}

In directly comparing people with CBS/Aβ+ve to those with CBS/Aβ-ve (Supplementary Table 2C and Table 2C), we confirmed higher $[^{18}\text{F}]$AV-1451 uptake across the cortical mantle, with more extensive volumetric loss particularly within the temporal-parietal-occipital subregions and the right caudate in CBS/Aβ+ve patients. Despite this however, the CBS/Aβ+ve cohort had less severe synaptic loss within both cortical and subcortical areas (Supplementary Table 1C, Table 2C). In other words, the pattern of synaptic loss and tau pathology distinguished CBS/Aβ+ve versus CBS/Aβ-ve cohorts. $[^{11}\text{C}]$UCB-J binding was preferentially reduced in posterior cortical areas in CBS/Aβ+ve patients, and more severely lost in anterior cortical and subcortical areas in the CBS/Aβ-ve group. Our results are in line with previous $[^{18}\text{F}]$FDG PET studies in CBS, reporting distinct patterns of glucose hypometabolism in amyloid-positive versus negative CBS, and pathologically confirmed CBS-AD, CBS-CBD, and CBS-PSP.\textsuperscript{12, 13, 15, 51} $[^{18}\text{F}]$FDG PET is often interpreted as a surrogate of synaptic density. However, our results go further to understand CBS heterogeneity without some of the confounds of $[^{18}\text{F}]$FDG PET (e.g. inflammation\textsuperscript{52}, and functional activation differences); and with the benefit of greater mechanistic specificity of the ligand.

Combining imaging and blood biomarkers in CBS can provide more accurate ante mortem diagnosis of different aetiologies, as well as help in understanding the distinct mechanistic pathways leading to a common set of clinical symptoms in this cohort. That the extent of synaptic loss is more severe in CBS/Aβ-ve despite modest grey matter volume loss compared to CBS/Aβ+ve, is an interesting finding. It has been shown before in preclinical models\textsuperscript{53} and post mortem studies,\textsuperscript{27} that synaptic loss can lead ahead of cell death and volume loss, and be very closely associated with clinical deficits in tauopathies. Our data do not answer why it is that this difference is more marked in CBS/Aβ-ve cases than CBS/Aβ+ve. We speculate that there is differential synaptotoxicity of 4R tau (likely present in amyloid-negative CBS) than the mixed 3R/4R tauopathy (likely found in CBS-AD).\textsuperscript{54} This differential toxicity might also account for the shorter average survival of patients with 4R tauopathies.\textsuperscript{55} Another explanation
for the differential patterns of synaptic loss, could be the likely heterogeneous distribution and progression patterns of tau pathology in CBS-AD versus CBS-CBD/PSP. While current blood biomarkers such as pTau217 levels or glial fibrillary acidic protein provide high specificity in differentiating between AD and non-AD dementias,\textsuperscript{8, 56} they do not reveal changes in specific pathological pathways or regions, but rather complement and enrich PET insights.

There are several limitations to this study. First, our sample size is modest limiting our ability to perform robust classification algorithms for each imaging modality. Despite this however, we observe moderate/large effect sizes for many of the between and within group comparisons. The Bayesian tests complement the more common frequentists tests, but provide added value in the ability to provide evidence in favour of the null (that there is no group difference), rather than merely a failure to reject the null. Second, in PET studies of neurodegeneration with atrophy, grey matter volume loss can affect the interpretation of PET signals. We used partial volume correction to minimise the effect of atrophy on binding estimates. However, the analyses without partial volume correction yielded similar results in all the main analyses. Third, we used only T1-weighted MRI as a structural marker. Other measures of structural integrity such as diffusion-weighted imaging may provide additional differentiation of the molecular aetiologies of CBS.\textsuperscript{57} Fourth, we classify our participants according to clinical diagnostic criteria and an arbitrarily thresholded $\beta$-amyloid SUVR value. Whereas we interpret that $\beta$-amyloid status as indicating likely underlying pathology of AD vs CBD/PSP pathology, this is not confirmed. Other pathologies may also give rise to the corticobasal syndrome,\textsuperscript{7, 58, 59} and co-pathology may also occur that would give rise to a positive $\beta$-amyloid scan even in someone with CBD.\textsuperscript{60} Lastly, the cross-sectional design of this study limits the interpretation of the dynamic relationship between pathology and synaptic loss. Although we include patients at various stages and severity of illness, a longitudinal design is necessary to test the dynamic relationship between synaptic loss and grey matter atrophy as disease progresses.

In conclusion, the amyloid status of people with corticobasal syndrome has a marked influence on the severity and distribution of synaptic and grey matter volume loss. Current clinicopathological correlations are poor, with Alzheimer’s disease causing 30-50% of corticobasal syndrome and corticobasal degeneration comprising the majority of non-AD aetiologies. We do not advocate $[^1\text{C}]$UCBJ PET as a diagnostic tool. Rather, it reveals the importance of severe synaptic loss in people with likely CBD, which we hope will inform future therapeutic strategies and improve future clinical trials design.
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Author contributions

NH, JTO and JBR contributed to the conception and design of the study. NH was involved in data acquisition, analysis, drafting the manuscript, and prepared the figures and tables. PSJ, GS, MN, MM, DJW, DS, PS, TDF, YTH, TR, contributed to data acquisition, and edited the final version of the manuscript draft. FIA, EM, KB, JTO and JBR edited the final version of the manuscript.

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Figure legends

Figure 1. Laterality index for $^{[11}C]UCB$-J $BP_{ND}$ (partial volume corrected) in aggregate brain regions. Negative values denote left-sided, and positive values right-sided predominant synaptic loss on PET imaging. Results are colour coded by the patient’s clinically most affected side. For those participants with predominantly left-sided symptoms (red), synaptic loss was more severe on the right (and ipsilateral cerebellum), and vice versa for those with right-sided predominant symptoms (green).

Figure 2.

(A) t-statistic brain maps illustrating regional differences in $^{[18}F]AV-1451$ and $^{[11}C]UCB$-J $BP_{ND}$ (both partial volume corrected) and grey matter (GM) volume for the following contrasts: Controls (Ctrl) more-than CBS $\beta$-amyloid positive (CBS/$\beta$+ve) (left-hand column), Controls more-than CBS $\beta$-amyloid negative (CBS/$\beta$-ve) (middle column), and CBS/$\beta$+ve more-than CBS/$\beta$-ve (right-hand column). Only t values, significant at $p<0.05$ corrected for multiple comparisons, are shown here. Higher t values for $^{[18}F]AV-1451$ represent higher binding in CBS/$\beta$+ve and CBS/$\beta$-ve compared to controls (dark blue) and in CBS/$\beta$+ve compared to CBS/$\beta$-ve (orange-red). Higher t values for $^{[11}C]UCB$-J regional comparisons, illustrate greater synaptic density in Controls versus both patient cohorts and, greater synaptic density in CBS/$\beta$+ve versus CBS/$\beta$-ve. Higher t values for volumetric comparisons show extensive grey matter volume loss in both patient cohorts compared to controls, with more severe loss in CBS/$\beta$+ve.

(B) Scatterplot showing a negative correlation between $^{[18}F]AV-1451$ and $^{[11}C]UCB$-J binding (both partial volume corrected) in 78 brain subregions, averaged across CBS/$\beta$+ve (blue) and CBS/$\beta$-ve (yellow) patients. Values are regional z-scores calculated against controls. Pearson correlation coefficient in CBS/$\beta$+ve: $R = -0.49$ ($p < 0.001$), and in CBS/$\beta$-ve: $R = -0.36$ ($p = 0.001$). Cortical regions (+), subcortical regions (O).