Synaptic loss in the primary tauopathies of Progressive Supranuclear Palsy and Corticobasal Degeneration

Negin Holland

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PREFACE

This thesis is the result of my own work and includes nothing which is the outcome of work done in collaboration except as declared in the preface and specified in the text. It is not substantially the same as any work that has already been submitted before for any degree or other qualification except as declared in the preface and specified in the text. It does not exceed the prescribed word limit for the Degree Committee of the Faculties of Clinical Medicine and Clinical Veterinary Medicine.

Some of the material presented in chapter 1 (pertaining to patterns of atrophy in Progressive Supranuclear palsy and Corticobasal syndrome) are as a result of collaborative research with the PROSPECT-UK consortium, and has been published as:

Jabbari, E., **Holland, N.,** Chelban, V., Jones, P.S., et al. 2019. Diagnosis Across the Spectrum of Progressive Supranuclear Palsy and Corticobasal Syndrome. JAMA Neurol. https://doi.org/10.1001/jamaneurol.2019.4347

Part of Chapter 3 has been published as:
Holland N, Jones PS, Savulich G, et al. Synaptic Loss in Primary Tauopathies Revealed by [^{11}C]UCB-J Positron Emission Tomography. Mov Disord. 2020;35(10):1834-1842. doi:10.1002/mds.28188

Parts of the material in chapter 4 is as a result of collaborative research and published as:
Mak, E*, **Holland, N*,** Jones, S, et al. In vivo coupling of dendritic complexity with presynaptic density in primary tauopathies. Neurobiol Aging. 2021. doi: 10.1016/j.neurobiolaging.2021.01.016 ***Joint first author**

Much of the material in Chapter 5 is under revision as:
Holland N, Malpetti M, Rittman T, Mak E, et al. The relationship between molecular pathology and synaptic loss in the primary tauopathies: a combined [^{18}F]AV-1451 and [^{11}C]UCB-J PET study.

Other relevant publications referenced within the thesis include:
Rowe JB, **Holland N**, Rittman T. Progressive Supranuclear Palsy: Diagnosis and Management. Practical Neurology. 2021. *In press.*

Holland N, Robbins TW, Rowe JB. The role of noradrenaline in cognition and cognitive disorders. Brain. 2021. doi: 10.1093/brain/awab111

Nicastro N, **Holland N**, Savulich G, et al. [^{11}C]UCB-J synaptic PET and multimodal imaging in dementia with Lewy Bodies. Eur J Hybrid Imaging. 2020. doi: 10.1186/s41824-020-00093-9

SYNAPTIC LOSS IN THE PRIMARY TAUOPATHIES OF PROGRESSIVE SUPRANUCLEAR PALSY AND CORTICOBASAL DEGENERATION.

Dr Negin Holland

In this thesis I address the debilitating symptom of cognitive dysfunction in the primary tauopathies of Progressive Supranuclear Palsy (PSP) and Corticobasal Degeneration (CBD). Both PSP and CBD are associated with an accumulation of 4-repeat tau in cortical and subcortical areas. As well as movement disorders, they impair cognitive function, even where there is minimal atrophy. Neurophysiological studies have also identified electrophysiological changes associated with cognitive dysfunction, in areas without atrophy. I propose that synaptic loss prior to cell loss contributes to these effects of disease.

Chapter two summarises my cohort and principal methods. I quantify synaptic density *in vivo* with dynamic [^{11}C]UCB-J PET, and molecular pathology with [^{18}F]AV1451 PET. Brain structural changes are quantified by MRI. Disease severity and cognition are assessed with the PSP rating scale, and neuropsychological tests. Patients with CBD are negative on amyloid-imaging ([^{11}C]PiB PET) to exclude those with Alzheimer's pathology. In chapter three, [^{11}C]UCB-J PET reveals widespread loss of synapses in PSP and CBD including areas with minimal atrophy. The loss of synapses correlated with cognition and disease severity.

In chapter four, I test whether presynaptic changes (from [^{11}C]UCB-J PET) are correlated with postsynaptic abnormalities (i.e. changes to postsynaptic dendritic microstructural integrity quantified by MRI using the Neurite Orientation and Dispersion Index, NODDI). In accordance with *in vitro* and animal models, I confirm that loss of dendritic complexity is tightly coupled with presynaptic density, over and above the effects of atrophy.

In chapter five, I test the relationship between the molecular pathology in primary tauopathies (tau burden) and synaptic loss, using [^{18}F]AV-1451 and [^{11}C]UCB-J PET. The use of the "tau" ligand [^{18}F]AV-1451 has become controversial in PSP. With due consideration to the caveats, I report that brain regions with a higher synaptic density have higher [^{18}F]AV-1451 binding, consistent with the hypothesis of connectivity-based

progression of tauopathy. I further show that accrual of pathology in any given area is associated with loss of synapses, consistent with synaptic injury from tauopathy.

I conclude my thesis in chapter 6, by discussing and highlighting the importance of synaptic density in primary tauopathies. The findings are relevant to other neurodegenerative disorders, and support early interventional studies targeting synaptic maintenance and restoration.

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Although I use the singular pronouns “I” and “my” throughout my thesis, this work would not have been possible without the tremendous support, effort and patience of my family, colleagues, collaborators, advisors, and supervisor.

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Neither my PhD, nor the write-up of this thesis, would be possible without the tireless support, encouragement and understanding from my parents, in-laws, and husband – to all of them I am eternally grateful.

DEDICATION

To my son and daughter, Daniel and Miriam.

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ABBREVIATIONS

Aβ	Amyloid β protein/protein residues; used as a diagnostic marker of Alzheimer's disease
ACE-R	Addenbrooke's Cognitive Examination – revised
AD	Alzheimer's disease
ANOVA	Analysis of variance
ARSAC	The Administration of Radioactive Substances Advisory Committee
[^{18}F]AV-1451	The tau PET tracer developed by Siemens Medical Solutions USA, Inc. and acquired by AVID and Eli Lilly and Company
BADL	Bristol Activities of Daily Living
BP_{ND}	Non-displaceable binding potential
BRC	Cambridge Biomedical Research Centre
bvFTD	Behavioural variant Frontotemporal dementia
CBD/CBS	Corticobasal Syndrome/Degeneration
CDR	Clinical Dementia Scale
CSF	Cerebrospinal fluid
CUH	Cambridge University Hospitals NHS Foundation Trust
DLB	Dementia with Lewy bodies
FTD	Frontotemporal dementia (including semantic dementia, Pick's disease, frontotemporal lobar degeneration, behavioural variant FTD, and several other variants)
FTLD	Frontotemporal Lobar Degeneration
LBD	Lewy Body Dementia
LME	Linear mixed effect model
MAPT	Microtubule associated protein Tau
MCI	Mild cognitive impairment
MHRA	Medicines and Healthcare products Regulatory Agency
MMSE	Mini mental state examination
MoCA	Montreal Cognitive Assessment
MRI	Magnetic resonance imaging
NHS	National Health Service
NIHR	National Institute for Health Research
PD	Parkinson's disease.
PDD	Parkinson's disease dementia.
PET	Positron emission tomography
[^{11}C]PiB	Pittsburgh compound B ([N-methyl-^{11}C]2-(4'-methylaminophenyl)-6-hydroxybenzothiazole), a radioactively labelled compound that binds to Aβ
PrEPPAReD	Prospective Evaluation of Parkinson's Plus And Related Disorders
PROSPECT-M-UK	Progressive Supranuclear Palsy, Corticobasal Syndrome and Multisystem Atrophy - a national cohort study.
PSP	Progressive Supranuclear Palsy
PSPRS	PSP Rating Scale
REC	Research ethics committee
SPM	Statistical parametric mapping - a technique used in analysis of MRI and PET data.
SEADL	Schwab and England Activities of Daily Living Scale
SENDeR	Synaptic Evaluation in Neurodegenrative Research
SUVR	Standard Uptake Value Ratio - used in PiB PET imaging
TENDeR	Tau Evaluation in Neurodegenerative Research
[^{11}C]UCB-J	((*R*)-1-((3-(methyl-^{11}C)pyridin-4-yl)methyl)-4-(3,4,5-trifluorophenyl)pyr-rolidin-2-one); synaptic vesicle 2A specific PET radioligand
WBIC	Wolfson Brain Imaging Centre (University of Cambridge)

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1 GENERAL INTRODUCTION

Summary

Neurodegenerative diseases are a major global cause of morbidity and mortality. A common symptom experienced by many patients with neurodegenerative disease is cognitive dysfunction (or dementia) manifesting as impairment in previously attained functioning – it can present with difficulties with memory, language, attention, and executive function. These symptoms are debilitating for patients, their carers and families, and result in great financial and personal cost (Winblad et al., 2016). Yet attempts to effectively treat or cure cognitive dysfunction in neurodegeneration, although promising (Boxer et al., 2019; Panza et al., 2019), has proven challenging. This has been in part due to the challenges in understanding the multifaceted underlying pathology: from genetic and environmental risk factors, accumulation and spread of misfolded proteins leading to dysfunction of neuronal communications and oscillations, and neurotransmitter deficits, with ensuing neuronal loss and loss of functional brain network connectivity – each of these processes contribute to cognition and affect survival both directly and synergistically. Of note however, cognitive symptoms can occur early in the disease process and neuronal loss is not essential to symptom manifestation. Indeed, in Alzheimer's disease, the loss of the core units of communication within the central nervous system – the synapse, correlates better with cognitive dysfunction than neuronal loss (Masliah et al., 1992; Scheff et al., 2006; Terry et al., 1991).

Synaptic loss is a common, early pathological process in neurodegeneration (Hamos et al., 1989), and occurs in response to misfolded proteins (Jacobsen et al., 2006), resulting in neuronal dysfunction before neuronal loss (Kaniyappan et al., 2017; Menkes-Caspi et al., 2015). Targeting this pathway in clinical trials may therefore provide an opportunity to modulate cognition and other physical symptoms early in neurodegeneration.

In this chapter, I introduce the main disease focus of my thesis – primary tauopathies - as a disease model for studying synaptic dysfunction. I review the pathological and clinical phenotypes of primary tauopathies and the common symptom of cognitive dysfunction, focusing in particular on Progressive Supranuclear Palsy and Corticobasal Degeneration. I then review the neural mechanisms underlying cognitive dysfunction, and emphasise the critical and central role of synapses as evidenced by animal models, post mortem and *in vivo* assessments. I summarise this chapter by setting out the hypotheses for each subsequent experimental chapter. Firstly, I propose that there is significant synaptic loss *in vivo*, in the primary tauopathies of Progressive Supranuclear Palsy and Corticobasal Degeneration correlating with cognition. Secondly, I hypothesise that the distribution of the underlying pathology in primary tauopathies correlates with synaptic loss, but that this relationship may be moderated by disease severity. Overall, I propose a key role of synapses in primary tauopathies and the associated cognitive dysfunction that is applicable to other neurodegenerative disorders, and a potential therapeutic target in future intervention studies.

1.1 PRIMARY TAUOPATHIES

1.1.1 PATHOLOGICAL PHENOTYPES ASSOCIATED WITH PRIMARY TAUOPATHIES

Primary tauopathies encompass a family of pathologies that are associated with frontotemporal lobar degeneration (FTLD) and the intracellular accumulation of the misfolded and hyperphosphorylated tau protein. The distinct pathological phenotypes are differentiated by the anatomical location of the abnormal tau protein, the specific cell types affected (astrocytes, oligodendrocytes, neurons), and the distinct tau isomers in the protein deposit (Kovacs, 2015). The pathological phenotypes include pure 4-repeat tauopathies: progressive supranuclear palsy (PSP), corticobasal degeneration (CBD), globular glial tauopathy (GGT), argyrophilic grain disease (AGD), and rarely associated with frontotemporal lobar degeneration associated with mutations in the microtubule associated protein tau (FTLD-MAPT; which can also present as a pure 3-repeat tauopathy and a mixed 3,4-repeat tauopathy); pure 3-repeat tauopathies: Pick's disease; and mixed 3 and 4-repeat

tauopathies: primary age-related tauopathy (PART) and neurofibrillary tangles dementia (NFT) (Figure 1-1).

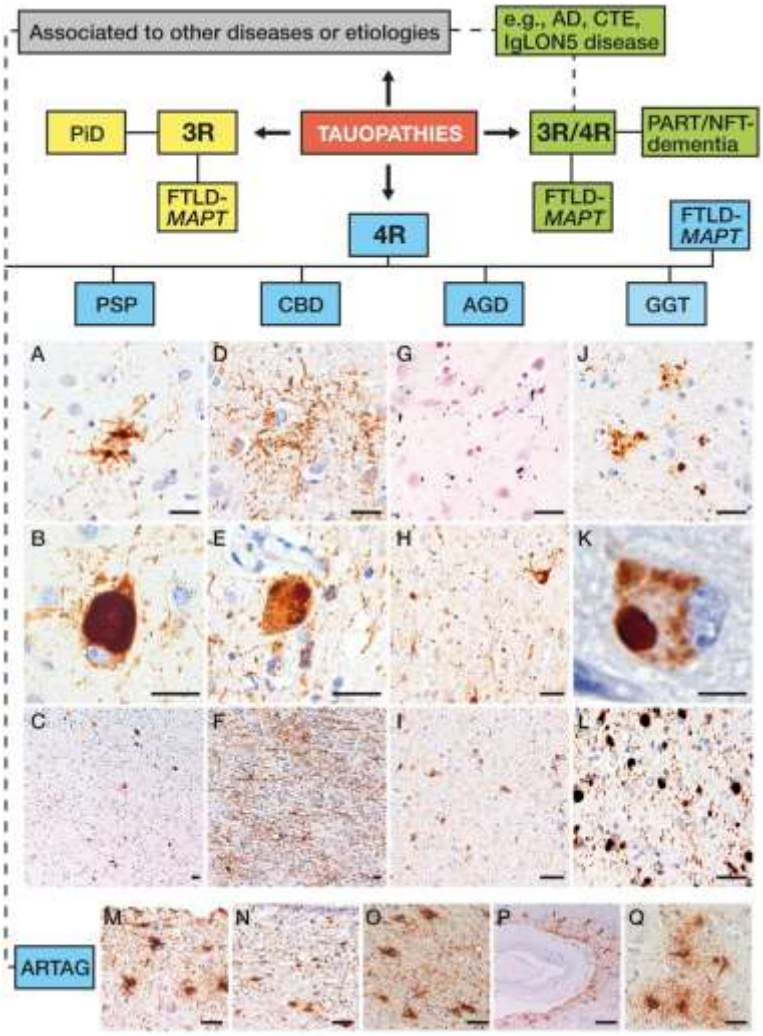

Figure 1-1. Molecular pathological classification of primary tauopathies.

Tauopathies are characterised by the predominant aggregation of tau isoforms with 3 repeats (3R), or 4 repeats (4R) of the microtubule-binding domain, or mixed 3R/4R isoforms. Secondary tauopathies are associated with other diseases or aetiologies (dashed lines; these include e.g. Alzheimer's disease (AD), chronic traumatic encephalopathy (CTE) or anti-IgLON5-related tauopathy). Primary tauopathies are pure in nature and are rarely associated with other proteinopathies but concomitant co-pathologies may exist. Pathological slices in this figure represent molecular pathological characteristics, evidenced by anti- phospho-tau antibodies of the following tauopathies: Progressive supranuclear palsy (PSP): tufted astrocyte (A), globose neurofibrillary tangle in the caudate nucleus (B), oligodendroglial coiled bodies in the internal capsule (C); corticobasal degeneration (CBD): astrocytic plaque in the frontal cortex (D), neuronal corticobasal bodies in the substantia nigra (E), threads and coiled bodies in the parietal white matter (F); argyrophilic grain disease (AGD): classical Gallyas-positive argyrophilic grains (G), tau-positive grains and neuronal cytoplasmic immunoreactivities (pre- tangles) in the hippocampus (H), coiled bodies in the hippocampal white matter (I); glial globular tauopathy (GGT): globular astroglial inclusions (J), spherical neuronal inclusions in the frontal cortex (K), globular oligodendroglial inclusions in the hippocampal white matter (L). Aging-related tau astrogliopathy (ARTAG): subpial (M), subependymal (N), white matter (O), perivascular (P) and grey matter (Q) astrogliopathy. Reproduced from Rösler *et al.* 2019, with permission from Elsevier.

1.1.2 CLINICAL PHENOTYPES ASSOCIATED WITH PRIMARY TAUOPATHIES

The clinical phenotypes associated with primary tauopathies are heterogenous, and manifest as movement disorders, cognitive disorders or commonly a mixture of both.

The clinical syndromes associated with pathologies of frontotemporal lobar degeneration (FTLD, Figure 1-2), include behavioural variant frontotemporal lobe dementia (with or without motor neuron disease), the primary progressive aphasias (including the non-fluent, semantic and logopenic variants), progressive supranuclear palsy and corticobasal syndrome (Coyle-Gilchrist et al., 2016; Gorno-Tempini et al., 2011; Murley et al., 2020a), all presenting with cognitive dysfunction as well as, in some cases, a movement disorder.

Cognitive dysfunction, in the form of personality change, executive dysfunction, impulsivity, apathy, and language deficits, are common in FTLD-related syndromes and debilitating for both patients and their carers (Burrell et al., 2014; Coyle-Gilchrist et al., 2016; Murley et al., 2020a; Piguet et al., 2011; Rascovsky et al., 2007). Yet the management of these disabling symptoms remains a challenge for clinicians. This is partly due to the multi-faceted underlying neural mechanisms, causing the clinical symptoms. These interlinked pathological entities include genetic/epigenetic (Ferrari et al., 2019; Höglinger et al., 2011; Wen et al., 2021; Yokoyama et al., 2017), and environmental risk factors (Litvan et al., 2016; Litvan et al., 2021), the aggregation and spread of misfolded proteins (Goedert et al., 2017a), alterations to neuronal physiology (Adams et al., 2021; Hughes et al., 2013; Sami et al., 2018) and chemical balance (Murley & Rowe, 2018), and the ensuing neuronal loss and functional brain connectivity (Cope et al., 2018; Gardner et al., 2013). Neuronal loss is however, a late phenomenon in neurodegeneration and electrochemical disturbances as well as communication breakdown, occur early with a better correlation with clinical symptoms. In this thesis, I focus on the concept of communication loss as a key neural basis for cognitive dysfunction in the primary tauopathies of progressive supranuclear palsy and corticobasal syndrome.

Progressive supranuclear palsy (PSP) and corticobasal syndrome (CBS), are primary tauopathies that were first described by Richardson, Steele and Olszweski (Steele et al., 1964), and Rebeiz and colleagues (Rebeiz et al., 1968), respectively. Our understanding of the pathophysiology of these two conditions has substantially increased since their original description in the 1960s. Through advancements in cryo-electron microscopy, we now

know the structure of the underlying pathogenic 4-repeat tau species (Scheres et al., 2020; W. Zhang et al., 2020); genome wide association studies have identified several risk loci, including the Microtubule Associated Protein Tau (MAPT)-H1 haplotype as a shared pathogenic risk factor for both PSP and CBS (Di Maria et al., 2000); and neuroimaging has helped in understanding the macrostructural, molecular and longitudinal changes through the disease course. Below I review the clinical features seen in PSP and CBS, with a particular emphasis on the cognitive profile and the potential underlying neural mechanism underlying cognitive dysfunction in these conditions.

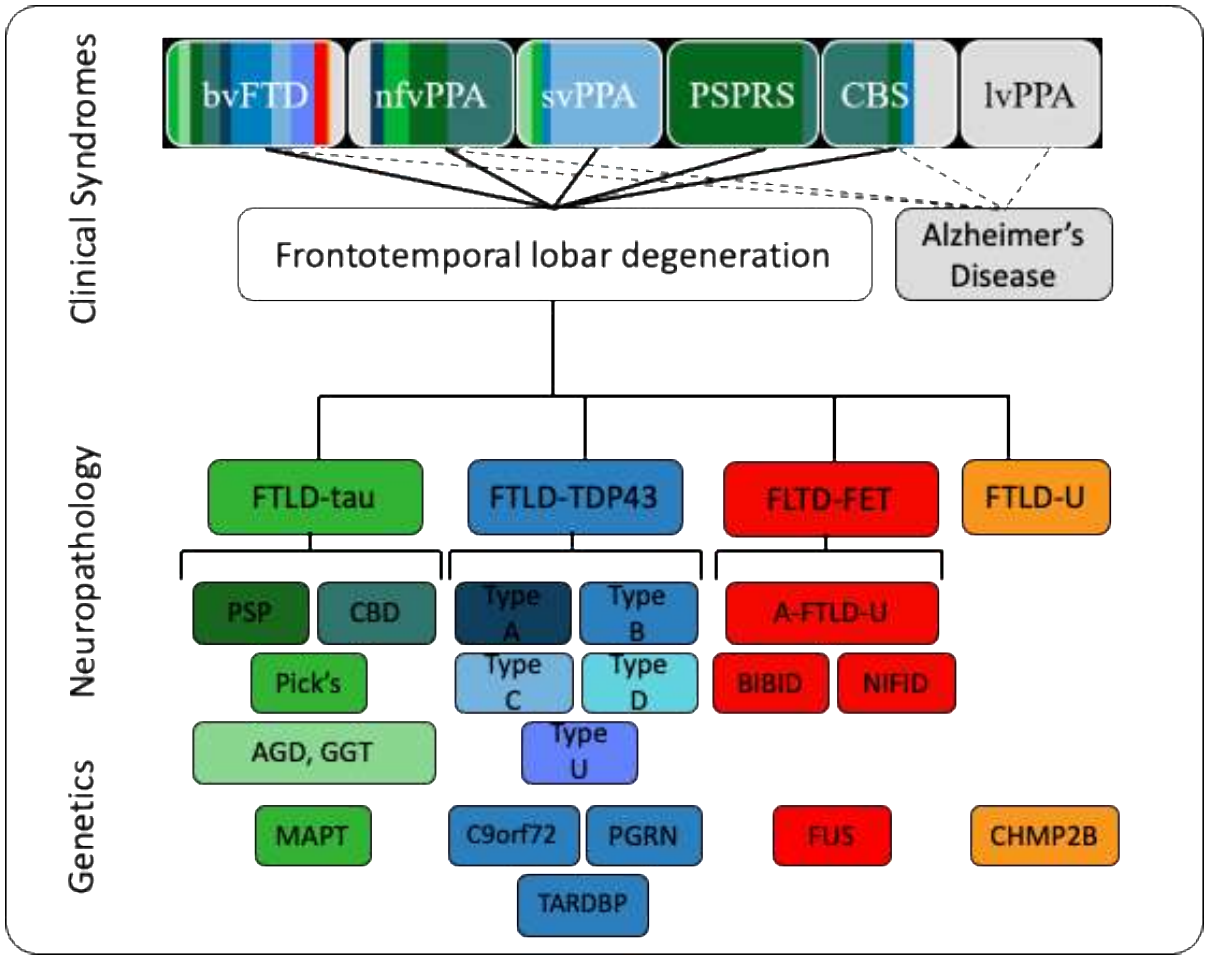

Figure 1-2. Clinicopathological spectrum of frontotemporal lobar degeneration syndromes including primary tauopathies.
The clinical syndromes caused by frontotemporal lobar degeneration (FTLD), coloured by the prevalence of each FTLD subtype: bvFTD: behavioural variant frontotemporal dementia; nvfPPA: non-fluent variant primary progressive aphasia; svPPA: semantic variant primary progressive aphasia; PSP: progressive supranuclear palsy-Richardson's syndrome; CBS: corticobasal syndrome; lvPPA: logopenic variant primary progressive aphasia. **Neuropathological subtypes of FTLD***:* PSP: progressive supranuclear palsy. CBD: corticobasal degeneration. AGD: argyrophilic grain disease. GGT: globular glial tauopathy. FTLD-FET: Fused in sarcoma (FUS), Ewing's sarcoma (EWS), and TAF15 proteins; FTLD-U: ubiquitin positive inclusions; BIBD: basophilic inclusion body disease. NIFID: neuronal intermediate filament inclusion disease. **Possible genetic mutations underlying FLTD:** MAPT: microtubule-associated protein tau; PGRN: progranulin; TARDBP: TAR DNA-binding Protein; FUS: fused in sarcoma; CHMP2B: charged multivesicular body protein 2b. Figure idea taken, and adapted from Professor William Seely, UCSF.

1.1.3 PROGRESSIVE SUPRANUCLEAR PALSY - CLINICAL SYMPTOMS AND SIGNS

Progressive supranuclear palsy (PSP) is a rare neurocognitive disorder with a prevalence of 10 per 100,000 (Coyle-Gilchrist et al., 2016). It was first described by Richardson, Steele and Olszweski (Steele et al., 1964) and initially clinically characterised by a vertical supranuclear gaze palsy, early postural instability and falls. This classic phenotype of PSP is referred to as Richardson's syndrome (PSP-RS), but it does not capture one of the main debilitating symptoms of PSP, namely cognitive dysfunction. Indeed, of the first nine patients described by Richardson, Steele and Olszweski, seven had significant cognitive dysfunction or dementia. The more recent diagnostic criteria, published in 2017, recognises PSP as a spectrum with features of movement abnormalities, visual and language difficulties, and cognitive dysfunction (Höglinger et al., 2017) (Supplementary Figure A1). In PSP, difficulties with movement are often due to a combination of global akinesia and rigidity, particularly around the neck and axial muscles, giving patients the typical hyperextended posture. Visual disturbance in PSP is a common symptom, presenting as slow and irregular vertical saccadic eye movements in early disease (Shaikh et al., 2017), and restricted eye movements overcome with the vestibulo-occular reflex, as the disease progresses (Anderson, 2015). Visual disturbance also occurs secondary to reduced blink rate (Bologna et al., 2009), lack of coordination (apraxia) of eye-lid opening or closing, and eye-lid spasms (blepharospasm) (Yoon et al., 2005). Postural instability is yet another common symptom, presenting within two to three years of symptom onset (Höglinger et al., 2017). Together with visual disturbance and movement abnormalities, postural instability leads to frequent falls in PSP but impulsivity and impaired decision making also heavily contribute (reviewed in Brown et al. (2020)).

Both speech and language are affected in PSP. Patients characteristically present with a slow, and dysarthrophonic speech, combined with hypokinetic, spastic and ataxic elements (Kluin et al., 2001; Rusz et al., 2015; Tykalova et al., 2017). The commonest language impairment is reduced fluency, but deficits in comprehension, and naming are also seen (Burrell et al., 2014; Peterson et al., 2021). In addition to speech impairment, other bulbar presentations in PSP include swallowing difficulties (dysphagia), a predictor of survival in PSP, which requires early attention and careful management (Glasmacher et al., 2017).

A degree of behavioural and cognitive impairment occurs in almost all patients with PSP; and behavioural change is a strong predictor of survival (Glasmacher et al., 2017; Murley et al., 2021), but management remains a challenge (Rittman et al., 2016; Rowe et al., 2021). The spectrum of symptoms overlap with those seen in patients with frontotemporal lobe dementia (FTD) (Bak et al., 2010). Impairments span those affecting frontal executive function (impaired decision making and problem solving, impaired attention, and rigid thinking), action inhibition (impulsivity), motivation (apathy), and speed of thinking (bradyphrenia), to social cognitive deficits (including impaired emotion recognition, loss of social behaviours with abnormal eating habits, and lack of insight) (Bak et al., 2010; Burrell et al., 2014; Lansdall et al., 2017; Robbins et al., 1994).

1.1.4 CORTICOBASAL SYNDROME - CLINICAL SYMPTOMS AND SIGNS

Cortricobasal syndrome (CBS) is a clinical diagnosis, first described by Rebeiz et al in 1968 (Rebeiz et al., 1968) (Figure A2). CBS is the commonest clinical presentation of the pathological diagnosis of the primary 4-repeat tauopathy of Corticobasal Degeneration (CBD), with a similar prevalence to PSP of 10 per 100,000 (Coyle-Gilchrist et al., 2016). The terms CBD and CBS have sometimes been used interchangeably, but this is no longer the case. Not all cases of CBD present with CBS; and not all people with CBS have CBD. The clinical features arising from CBD may overlap with frontotemporal lobe dementia, non-fluent variant primary progressive aphasia, or PSP (Rösler et al., 2019). Similar heterogeneity is seen in the underlying pathological diagnosis presenting with a CBS phenotype, including CBD, Alzheimer's disease (Alexander et al., 2014; Burrell et al., 2013; S. E. Lee et al., 2011), PSP (Josephs et al., 2006; Ling et al., 2010), and TAR DNA-binding Protein (TDP-43) (S. E. Lee et al., 2011) .

The clinical phenotype of CBS manifests with motor signs, language difficulties and cognitive dysfunction (Armstrong et al., 2013). Symptoms include asymmetric cortical and basal (subcortical) features (Supplementary Figure A2). Cortical symptoms include apraxia (affecting the eye lids, face, speech and limbs) (Zadikoff & Lang, 2005), alien limb phenomenon (Lewis-Smith et al., 2020), cortical sensory loss, and visuospatial dysfunction (simultagnosia and visual inattention) (Burrell et al., 2014; Rittman et al., 2013). Subcortical features include dystonia, rigidity, and akinesia. Dystonia in CBS can affect the limbs, neck or the eyelids and in some cases is responsive to dopaminergic medication

(Stamelou et al., 2012). Patients can also present with myoclonus (coarse tremor) featuring as either a subcortical (Grosse et al., 2003) or cortical phenomenon (Carella et al., 1997).

Speech production in CBS can be affected by apraxia of speech (orobuccal apraxia) but primary language deficits affecting comprehension, fluency and repetition are also seen (Burrell et al., 2014; Peterson et al., 2021; Rittman et al., 2013). Behavioural features in CBS overlap with those seen in PSP and frontotemporal lobe dementia, manifesting as apathy, impulsivity, and stereotyped movements (Burrell et al., 2014; Lansdall et al., 2017; Litvan et al., 1998).

On terminology of CBS/CBD, the current consensus is that CBS refers to the clinical syndrome whilst CBD refers to the characteristic 4-repeat tau pathology. About a third of those with CBS have Alzheimer's pathology (Alexander et al., 2014). In experimental chapters 3-5, I exclude this cohort of patients using amyloid imaging and use the term CBD to describe the group in whom amyloid imaging is negative.

1.1.5 NEURAL MECHANISMS OF COGNITIVE DYSFUNCTION IN PSP AND CBS

Many different pathological processes contribute to the cognitive deficits seen in PSP/CBS. At their core however, lies synaptic toxicity caused by the underlying pathology, and in turn leading to loss of structural and functional connectivity. I briefly review these pathological mechanisms below before expanding on pertinent aspects in chapters 3-5.

1.1.5.1 Tau Pathology

In a healthy brain, tau protein is abundant within neuronal axons (although it is also found in the somatodendritic compartment), where it serves as a key regulator of microtubular stability, neuronal dynamic and intracellular transport (V. M. Lee et al., 2001; Trojanowski et al., 1989; Y. Yoshiyama et al., 2013). Tau protein is encoded by the microtubule-associated protein tau (MAPT) on chromosome 17 and can form 6 different isomers through alternative splicing. The latter process determines how many repeat regions reside within the microtubule-binding domain of the protein, generating three isoforms each with three (3R) or four repeats (4R) (V. M. Lee et al., 2001).

Under normal conditions, tau can undergo a series of post-translational modifications including phosphorylation, acetylation and methylation (reviewed in Rösler et al. (2019)). Due to genetic and/or environmental risk factors, the normal unfolded tau protein can

become hyperphosphorylated, misfolded, and subsequently form soluble oligomers and then insoluble intracellular fibril aggregates in neurons and glial cells. The downstream effect of hyperphosphorylated tau is many fold: (1) within the axon it has reduced binding affinity for microtubules and other proteins therefore leading to an abnormal cytoskeleton, and ineffective intracellular molecular transport (Niewidok et al., 2016); (2) within the dendritic spine, it interferes with synaptic function (Frandemiche et al., 2014; Regan & Cho, 2019), and loses the ability to interact with other binding targets for example the plasma membrane or DNA (Ekinci & Shea, 2000; Hanger et al., 2009); and (3) within both neurons and glial cells it gains toxic function and the ability to propagate across the brain (Clavaguera et al., 2009; Goedert et al., 2017a; Mandelkow & Mandelkow, 2012). In addition to hyperphosphorylation, abnormal methylation can also contribute to tau pathology (Park et al., 2018; Weber et al., 2018).

Hyperphosphorylated tau is the principal pathology in primary tauopathies, unlike the secondary tauopathy of Alzheimer's disease where tau proteinopathy is secondary to beta-amyloid pathology (Selkoe & Hardy, 2016). Primary tauopathies can be mixed in their tau isoforms 3R/4R, predominant 3R, and predominant 4R.

The staging of the disease process in PSP depends on the anatomical areas affected, as well as the specific cell type harbouring hyperphosphorylated tau. Tau neurofibrillary tangles and threads, as well as tufted astrocytes, are seen initially within subcortical nuclei of patients with PSP before spreading cortically. Additionally, coiled bodies within oligodendroglial cells with diffuse cytoplasmic immunoreactivity within neurons, are also observed. With disease progression to fronto-parietal, temporal and occipital cortices, cortical astroglial tau accumulation ensues, followed by the involvement of cortical neuronal and oligodendroglial cells (Kovacs et al., 2020). In CBD, tau aggregates are seen in neurons and glial cells giving the appearance of coiled bodies and astrocytic plaques, with tau threads found within the white and grey matter. PSP and CBD tau filaments are subtly different in their biochemical features and distribution. Neuronal tau pathology is more pronounced in the forebrain in CBD, whilst PSP mainly affects hindbrain structures. Similarly, the degree of white matter involvement is greater in CBD than PSP, whilst subcortical neurofibrillary tangles are more pronounced in PSP (Rösler et al., 2019).

The density of cortical neurofibrillary tangles is strongly correlated with cognitive function, in PSP, CBS and Alzheimer's disease (Murray et al., 2014; Nelson et al., 2012), particularly affecting the executive cognitive domain (Koga et al., 2017). The mechanisms by which tau affects cognition are however multifaceted, and include both direct and indirect mechanisms with synaptic dysfunction as a key pathological consequence (Jadhav et al., 2015; Spires-Jones & Hyman, 2014). Direct pathways of tau-mediated synaptotoxicity include interference with dendritic morphology, synaptic protein expression, and synaptic vesicle numbers. Indirectly, tau adversely affects the functioning of the neuronal support network, including microglia cells and astrocytes, which in turn affect synaptic function (Kovacs et al., 2020; Vogels et al., 2019).

1.1.5.2 Genetic risk factors in PSP and CBS

Genetic risk factors, although not directly linked with cognitive outcomes in primary tauopathies, are linked to key pathogenic mechanisms that correlate with cognition and survival. For example, a recent Genome Wide Association Study (GWAS) in PSP has identified a single nucleotide polymorphism at loci rs2242367 associated with increased expression of pathogenic LRRK2 mutations, and decreased survival (Jabbari et al., 2021). Pathogenic mutations in LRRK2 lead to phosphorylation of a subset of Rab proteins (involved in synaptic vesicle trafficking) (Alessi & Sammler, 2018), therefore disrupting synaptic neurotransmitter release and proteostasis. Additionally, pathogenic LRRK2 interferes with actin and mitochondrial dynamics and with tau aggregate clearance, via the proteosome system. Consequently, although there is no direct link between LRRK2 risk alleles and cognition, the pathogenic mechanisms arising from mutations in this gene, synaptic transmission and tau accumulation and spread (Guerreiro et al., 2016; Islam et al., 2016; Lin et al., 2010), do cause cognitive dysfunction both in animal models, and human studies. An earlier GWAS identified genetic polymorphisms in the tripartite motif-containing protein 11 (TRIM11) associated with variations in PSP phenotype (PSP-Richardson's syndrome versus non-Richardson's syndrome including those patients with predominant cognitive features – PSP-frontal, PSP-CBS, and PSP-language (Höglinger et al., 2017; Jabbari et al., 2018) . TRIM11 is a component of the ubiquitin proteosome system and therefore central to the clearance of misfolded proteins (L. Chen et al., 2017); mutations may therefore interfere with the clearance of pathogenic tau species in primary tauopathies, consequently affecting cognitive symptoms.

1.1.5.3 Neuroinflammation

Glial cells are the immune cells of the central nervous system and are divided into microglia (macrophage cells, acting as the immune cells of the brain), and macroglia (oligodendrocytes, astrocytes, and ependymal cells). There is growing evidence for their role in PSP/CBD pathogenesis. Under normal physiological conditions, neuroinflammation within the central nervous system acts as a protective mechanism to maintain homeostatis; it is a controlled mechanism triggered by environmental factors, infection, toxic aggregates, and neuronal damage. Furthermore, a key role for glia cells, particularly astrocytes, in neural transmission has long been identified, such that many propose synaptic transmission to be dependent not only on the pre- and postsynaptic compartment but also the astrocytes. In this role, they are able to release neurotransmitters in response to neural activity, and therefore modulate synaptic function (Reid et al., 2020). PSP/CBD tau aggregates within astroglial cells, and in the extracellular space, disturb normal neuronal homeostasis, and co-localise with markers of inflammation at post mortem (Kovacs, 2020) and *in vivo* (Gerhard et al., 2006; Gerhard et al., 2004; Malpetti et al., 2020b). The mechanism by which inflammation causes functional impairment is again multifactorial with synaptic dysfunction as a key mechanistic player (reviewed in Vogels et al. (2019)).

1.1.5.4 Synaptic loss in primary tauopathies

Abnormal functioning of synapses and reductions of up to 50-60% in synaptic numbers have been observed in animal models of tauopathy (e.g. rTg4510) (Kaniyappan et al., 2017; Menkes-Caspi et al., 2015), in human post mortem brains (Bigio et al., 2001; Lipton et al., 2001), and proposed from measures of indirect effects of synaptic density and function via fluorodeoxyglucose (^{18}F) positron emission tomography, FDG-PET (Beyer et al., 2018), in both PSP and CBD. Loss of synaptic function and number is linked to the direct and indirect consequence of toxic tau oligomers and aggregates, as well as neuroinflammation (Jadhav et al., 2015; Spires-Jones & Hyman, 2014; Vogels et al., 2019). The downstream effect is that of interference with vesicular trafficking, disturbances in neurotransmitter release and the balanced electrochemical activities of a neuron, even before neuronal death occurs. This consequential effect is seen in both the presynaptic, and postsynaptic compartments. In animal models of tauopathy, and in post mortem studies, tau aggregates, as well as being concentrated within the cytoplasm are also present in dendritic processes (as well as in the extracellular compartment), subsequently interfering with synaptic transmission both

presynaptically and postsynaptically (Feany & Dickson, 1995; Herms & Dorostkar, 2016; Hoffmann et al., 2014; Hoover et al., 2010). The consequence of this, is disturbances in functional connectivity, and structural integrity. The effect of synaptic dysfunction and loss on cognitive function, has been investigated in animal models of tauopathy that mimic primary tauopathies (Menkes-Caspi et al., 2015), in post mortem brains of patients with the secondary tauopathy of Alzheimer's disease (Terry et al., 1991), and more recently directly using a novel PET radioligand [^{11}C]UCB-J (Finnema et al., 2016). Using the latter PET radiotracer has enabled the *in vivo* assessment of synaptic loss in mild cognitive impairment and Alzheimer's disease (M. K. Chen et al., 2018; Mecca et al., 2020), and Parkinson's disease and Lewy Body Dementia (Andersen et al., 2021; Matuskey et al., 2020; Nicastro et al., 2020), with significant correlations with the severity of cognitive symptoms. However, the direct *in vivo* evaluation of synaptic density and its relationship with cognition, in primary tauopathies, is yet to be determined.

1.1.5.5 Neurotransmitter deficits, and disturbed cortical physiology

A consequence of tau and inflammation mediated synaptic dysfunction, is disturbance in neurotransmitter release and therefore abnormal cortical physiology, in turn affecting cognition. Many key neurotransmitters for normal cognition are reduced in PSP, and CBS, albeit to a variable extent in each disease. These include noradrenaline, dopamine, γ-aminobutyric acid (GABA), glutamate, serotonin and acetylcholine (Holland et al., 2021; Murley & Rowe, 2018). Cortical physiology, as measured with magnetoencephalography is disturbed in PSP (Adams et al., 2021; Hughes et al., 2013; Sami et al., 2018). Impairment in baseline GABA levels in PSP has been identified as a key contributor to abnormal cortical physiology (Adams et al., 2021), and correlates with frontal executive dysfunction both directly, and indirectly (through interaction with glutamate) (Bastos et al., 2012; Murley et al., 2020b).

1.1.5.6 Structural and functional connectivity

The downstream effect of tau accumulation, inflammation, synaptic loss and abnormal cortical circuitry is neuronal loss and changes in structural and functional connectivity. Structural connectivity can be assessed using MRI. On volumetric MRI sequences, grey matter atrophy of the basal ganglia, midbrain, thalamus, and the cerebellum are common findings in PSP (J. L. Whitwell et al., 2017a). MRI assessment of midbrain atrophy is

particularly used in clinical practice, with atrophy here giving rise to the so called "hummingbird", "Mickey Mouse" and "morning glory" signs representing dorsal midbrain atrophy, rectangular midbrain peduncles, and the concavity of the midbrain tegmentum, respectively (Massey et al., 2012; C. Mueller et al., 2018). In PSP, frontal atrophy can affect the motor, premotor, orbitofrontal and medial frontal cortices. The degree to which this occurs depends on the dominant clinical phenotype – for example in patients with a more cortical presentation of PSP, as per the 2017 MDS criteria, including PSP-frontal, PSP-speech/language, or PSP-CBS overlap, frontal atrophy is more evident but it is present to some extent in most phenotypes during the course of the disease (Jabbari et al., 2019).

Frontal atrophy is also present in CBS, more prominent in the posterior frontal lobes (supplementary motor cortex, dorsal premotor, prefrontal cortex and anterior central gyrus). Atrophy in the anterior parietal lobes, central structures such as the cerebellum, caudate and thalamus are also seen, but may be initially asymmetric underlying the asymmetric clinical signs in CBS (Constantinides et al., 2019). The underlying pathology of CBS determines the distribution of atrophy, with those due to CBD and PSP pathology mimicking PSP patterns of atrophy. Frontal cortical atrophy in PSP is directly linked to cognitive function (Cordato et al., 2005; Cordato et al., 2002), however atrophy is rather a late phenomenon in the pathogenesis of PSP/CBS, and in neurodegeneration at large, as depicted schematically in Figure 1-3 (Sperling et al., 2011), and frontal executive dysfunction is present even when atrophy is minimal or absent.

A complementary way of assessing structural integrity is through Diffusion Tensor Imaging (DTI). In PSP, changes to white matter tracts seen on DTI, including the superior cerebellar peduncle, commissural and association fibres (superior longitudinal fasciculus, superior fronto-occipital fasciculus, cortico-cortical association fibres), and corticostriatal descending fibres are widely reported (Agosta et al., 2012; Crespi et al., 2020; Padovani et al., 2006; Jennifer L. Whitwell et al., 2011; Jennifer L Whitwell et al., 2021). In CBS, abnormal diffusion in anterior and posterior central gyri, middle frontal gyrus bilaterally, and superior and inferior frontal gyrus contralateral to the most clinically affected side have been reported (Boelmans et al., 2009; Bozzali et al., 2008; Jennifer L Whitwell et al., 2014). Reduced diffusion in the body of the corpus callosum, middle cingulum bundle, and the prefrontal cortices, and fronto-occipital fasciculus are common in both CBS and PSP (Jennifer L Whitwell et al., 2014). Abnormal diffusion is correlated with decline in

cognitive function, and clinical symptoms over time. For example, in PSP changes in the superior cerebellar peduncle integrity are correlated with rate of decline in the PSP rating scale (ocular sub-score), whereas decline in global cognition is correlated with changes in the post-central region and the mid-posterior cingulum. In CBS, rates of decline in the PSP rating scale (limb sub-score) are correlated with abnormal diffusion in the post-central white matter regions (Y. Zhang et al., 2016).

Functional connectivity in PSP and CBS is abnormal and can be measured using task-free functional MRI. Evidence thus far suggests increased within network connectivity, for example within the default mode network, cerebellum (Bharti et al., 2017), or within cortical areas (Cope et al., 2018). Connectivity between networks is however reduced, for example between the lateral visual and auditory resting state networks, and cortical-subcortical areas (Ballarini et al., 2020; Bharti et al., 2017; Cope et al., 2018; Gardner et al., 2013). This pattern of change in connectivity suggests a functional reorganisation in which local networks are strengthened, and long-distance connections are weakened, in response to disease. Changes in functional connectivity within the cerebellar resting state networks are correlated with measures of global cognition (Bharti et al., 2017).

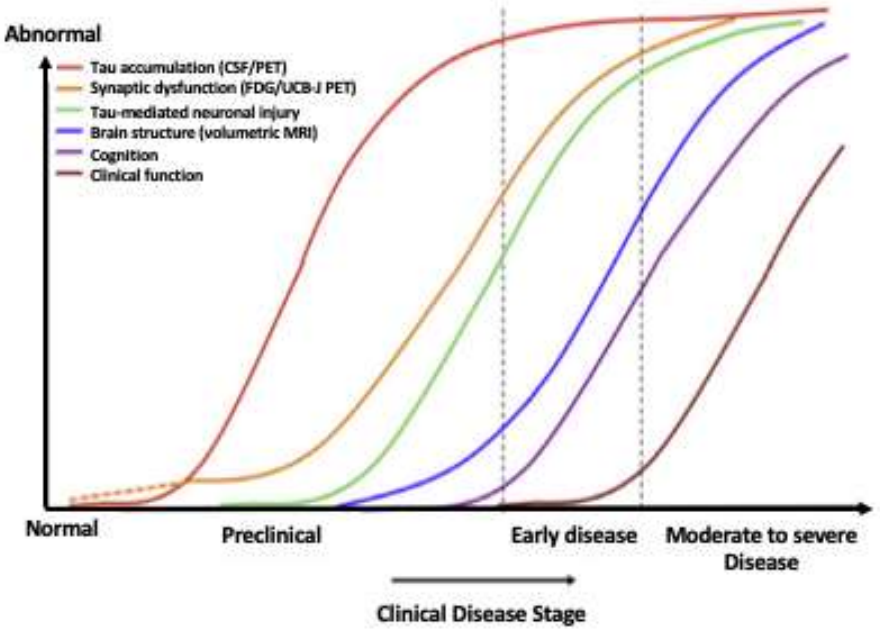

Figure 1-3. Theoretical model of dynamic changes to tauopathy related biomarkers in relation to clinical symptom manifestation.
This model emphasises the preclinical phase of tauopathies (but is applicable to other neurodegenerative disorders). Here synaptic dysfunction, shown in orange (indicated by metabolic changes measured by fluorodeoxyglucose positron emission tomography, FDG-PET, UCB-J PET or functional MRI) occurs before macro-structural change (volumetric MRI). Synaptic dysfunction may be detectable before detectable tau accumulation. Biomarkers change from normal to maximally abnormal (y-axis) as a function of disease stage (x-axis) – figure reproduced from Sperling et al 2011 with permission from Wiley.

1.2 Conclusion

In this chapter, I have provided an overview of the clinical syndromes associated with the primary tauopathies of PSP and CBD, and highlighted the importance of synaptic pathology as a contributor to functional changes (this topic will be covered in greater detail in Chapter 3). I therefore propose a critical role of synaptic density in cognition, and investigate this proposal in primary tauopathies, in this thesis. Throughout this thesis, I consider PSP and CBD as disease models of neurodegeneration given their high clinicopathological correlation with 4-repeat tauopathy at post mortem (where Alzheimer's pathology in the case of CBS has been eliminated, and referred to as CBD). In my experimental chapters I consider PSP and CBD together given the clinicopathological overlap but will highlight differences where relevant.

1.3 AIMS AND STRUCTURE OF THIS THESIS

This thesis aims to test the hypothesis that synaptic pathology plays a key role in primary tauopathies of PSP and CBD. In the experimental chapters, I use the novel positron emission tomography (PET) radioligand [^{11}C]UCB-J as a measure of *in vivo* synaptic density, and review the evidence for the use of this ligand in the introduction to chapter 3. In chapter 2, I summarise the clinical cohort, the research protocol, and the analysis techniques forming the basis of this thesis.

In chapter 3, I test the hypothesis that synapses are lost *in vivo* in PSP and CBD compared to healthy controls. To do this, I use [^{11}C]UCB-J PET as a measure of synaptic density, 3T MRI for structural brain volume analysis and, a neuropsychological battery for the assessment of cognition and disease severity. I predict a synaptic loss of 20-30% *in vivo*, considering there is up to 60% loss at post mortem. I further predict that this loss is beyond the degree of brain atrophy, and that it correlates with functional outcomes. Subsequently in chapter 4, I test whether *in vivo* changes in presynaptic density correlates with changes within the postsynaptic compartments. I test the degree of presynaptic synaptic loss with [^{11}C]UCB-J PET and changes within the postsynaptic compartment with Orientation Dispersion MRI (a measure of neurite complexity and a correlate for postsynaptic density). I predict that presynaptic loss as measured with [^{11}C]UCB-J PET, is positively correlated with changes in postsynaptic dendritic complexity.

To understand the contribution of tau pathology in PSP and CBD, in chapter 5, I test the hypothesis that synaptic density correlates with molecular pathology (using [^{18}F]AV-1451 PET). I predict that the relationship between the two processes will be complex and moderated by disease severity. I conclude this thesis by discussing the evidence for a critical role of synapses in neurodegeneration, and how this allows an opportunity for the development of future therapeutic targets.

2 General Methods

Summary

In this chapter I outline the general methods which underpin each experimental chapter in my thesis, in particular the selection and recruitment of participants, the neuropsychological assessment and neuroimaging aspects of the protocol, and the analysis techniques used (with more detail included in respective chapters). This study of synaptic pathology in primary tauopathies is observational in nature and is conducted within the overarching Synaptic Evaluation in Neurodegeneration Research (SENDeR), and Tau Evaluation in Neurodegeneration Research (TENDeR) protocols which form two major research themes of the Cambridge Centre for Parkinson-Plus.

2.1 RECRUITMENT

2.1.1 PARTICIPANTS

Patients with Progressive Supranuclear Palsy – Richardson's Syndrome (PSP-RS) (Höglinger et al., 2017), and possible or probable Corticobasal Syndrome (CBS) (Armstrong et al., 2013), were recruited into the study, as well as suitably matched healthy control participants defined by a Mini-Mental State Examination (MMSE) score greater than 26, and absence of regular memory complaints or signs/symptoms of dementia or unstable/ significant medical illness.

Patient participants were identified from clinics for cognitive and movement disorders within Cambridge University Hospitals and from neurology, and related services within other Trusts within the region including those in Cambridgeshire, Lincolnshire, Bedfordshire, Norfolk, Suffolk, Hertfordshire and Essex, and outside the region including London and Nottingham. Patient and control participants were also recruited from the National Institute for Health Research 'Join Dementia Research' (JDR) programme. JDR is an on-line self-registration service that enables volunteers with memory problems or dementia, carers of those with memory problems or dementia, and healthy volunteers to register their interest in taking part in research.

Potential participants so identified who expressed a willingness to take part in research were sent a copy of the study information sheet, together with the standard PET and MRI safety questionnaires. After a period to consider these materials (minimum 48h) they were contacted to inquire whether they were willing to take part, and to provide further information or allow further time for consideration as appropriate, as well as ensuring that they are likely to be suitable to take part in the study.

2.1.2 INCLUSION CRITERIA

Participants of both sexes, and above the age of 50 were included in the study and had sufficient proficiency in English to allow standardised cognitive testing. All patient participants had a reliable informant, who was able to complete questionnaires for informant-rated scales and provide background history. For patient participants, only those with mild to moderate dementia were included with MMSE $\geq$ 12, since subjects scoring lower than this were highly unlikely to be able to comply with study procedures.

2.1.3 EXCLUSION CRITERIA

Potential participants with concurrent major psychiatric illness (e.g. major depression), severe physical illness or co-morbidity that would limit ability to fully participate in the study were excluded, as well as those with contraindications to MRI safety (pacemaker, certain protheses, or claustrophobia). Potential participants were also excluded if they had atypical or focal parenchymal appearances on previous MRI which were not in keeping with their diagnosis, or which would interfere with neuroimaging processing (for example, excess white matter changes, or large areas of previous stroke). Potential participants taking concurrent medications that might have affected study assessments were also excluded - for example, the anti-epileptic medication levetiracetam (Lynch et al., 2004), which binds to the molecular target of the synaptic PET radioligand used in this study.

Eligible participants suitable to take part, provided written informed consent in accordance with the Deceleration of Helsinki, with patients being supported by their relative, spouse, close friends, or advocate when necessary.

2.2 ETHICAL & RADIATION APPROVALS

All PET radioligands were subject to prior approval from the Administration of Radioactive Substances Advisory Committee (ARSAC). The study protocol was approved by the Research Ethical Committee (18/EE/0059). All participants completed an approved MRI safety questionnaire to establish safety for scanning. PET scanning was undertaken within the safety procedures operating within the Wolfson Brain Imaging Centre (WBIC).

2.3 OVERVIEW OF STUDY PROTOCOL

Upon the provision of written consent, participants underwent a clinical and neuropsychological assessment as well as neuroimaging; the study procedure is set out in Figure 2-1. Most participants consented to having blood samples taken to measure a number of neurodegenerative biomarkers. A percentage of patient participants will be followed-up longitudinally, however this phase of the study as well as biomarker analysis lie outside the scope of my PhD.

2.3.1 NEUROPSYCHOLOGICAL AND CLINICAL ASSESSMENT

All participants underwent an initial clinical assessment, including the collection of basic clinical and demographic variables (e.g. symptoms pertaining to the neurodegenerative diagnosis, previous medical history, smoking/alcohol and family history). Participants completed a neuropsychological assessment using a test battery tailored according to the condition under study, which included measures of: pre-morbid ability, current activities of daily living, motor function and coordination, anxiety, depression and behavioural changes, visuospatial perception, working memory and attention, episodic memory, semantic memory, logic and reasoning, and verbal fluency. For the purposes of my thesis, the neuropsychological assessments used were the Addenbrooke's Cognitive Examination-Revised (ACE-R) (Mioshi et al., 2006), Mini-Mental State Examination (MMSE) (Arevalo-Rodriguez et al., 2015), Montreal Cognitive Assessment (MoCA) (Nasreddine et al., 2005), Ineco (frontal lobe assessment tool) (Torralva et al., 2009), and the Schwab and England Activities of Daily Living (SEADL) (Schwab & England, 1969). Patients' relatives also provided their report of the patient's functional abilities through completion of the Cambridge Behavioural Inventory (CBI) questionnaire (Wear et al., 2008) (Table 2-1). Disease severity was assessed using the clinician-rated PSP rating scale (Golbe & Ohman-Strickland, 2007), and Cortico Basal ganglia Functional Scale (CBFS) (Lang et al., 2020), where both scales are highly correlated and appropriate for use in both cohorts (Lang et al., 2020).

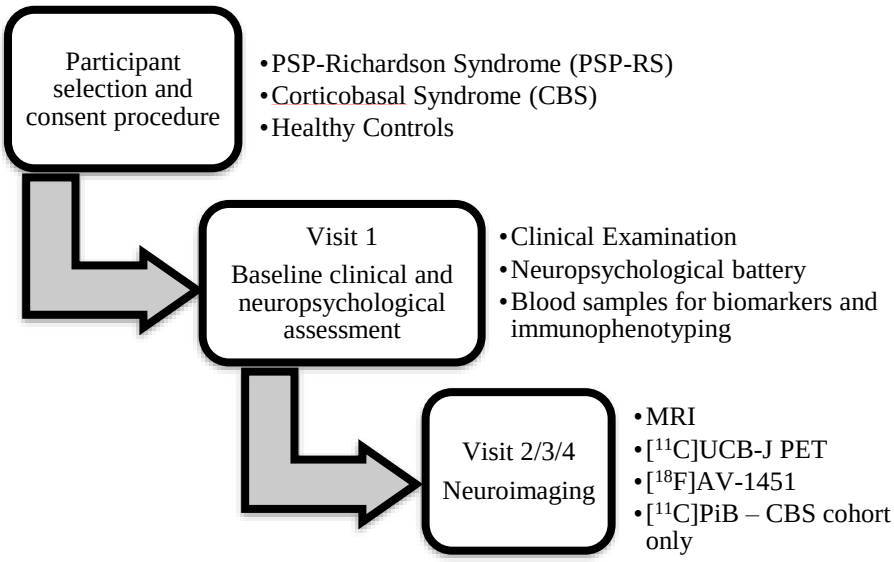

Figure 2-1. Flowchart illustrating the participants' journey through the study.

Assessment	Format	Purpose
Clinical		
Progressive Supranuclear Palsy Rating Scale (PSPRS)	Researcher administered structured assessment	Disease severity scale
Cortico Basal ganglia Functional Scale (CBFS)	Researcher administered structured assessment	Disease severity scale
Clinical Dementia Rating Scale (CDR)	Researcher rated	Overall impression of cognition
Neuropsychological		
Addenbrooke's Cognitive Examination-Revised (ACE-R)	Researcher administered structured assessment	Multi-domain cognitive screening tool
Mini-Mental State Examination (MMSE)	Researcher administered structured assessment	Multi-domain cognitive screening tool
Montreal Cognitive Assessment (MoCA)	Researcher administered structured assessment	Multi-domain cognitive screening tool
INECO Frontal Screening Tool	Researcher administered structured assessment	Frontal lobe assessment tool
Schwab and England Activities of Daily Living (SEADL)	Scale of 0-100%	Clinician's impression of participant's functional independence
Informant Questionnaires		
Cambridge Behavioural Inventory (CBI)	Carer-reported questionnaire	Assessment of several behavioural abnormalities in everyday life including impulsivity and apathy

Table 2-1. Clinical and neuropsychological assessment battery, and questionnaires

2.3.2 NEUROIMAGING

Participants underwent MRI scanning at baseline, as well as two PET scans ([^{11}C]UCB-J, and [^{18}F]-AV1451). Those participants with Corticobasal Syndrome (CBS), had an additional PET scan with [^{11}C]PiB to determine their amyloid status – those with a negative amyloid status (henceforth referred to as CBD) were carried forward for subsequent analysis in this thesis. All scanning sessions were carried out at the Wolfson Brain Imaging Centre (WBIC).

2.3.2.1 Magnetic Resonance Imaging

MRI scanning was performed on two separate occasions. One was acquired during PET data acquisition including a T1-weighted sequence specifically used for the purpose of co-registration with PET images. Here, T1-weighted images were acquired on a 3 Tesla GE

SIGNA PET/MR with the following parameters: repetition time (TR) = 3.6 msec, echo time (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). T1 images acquired here allowed tissue classification into grey and white matter and CSF and were rigidly registered to PET images; using a version of the Hammersmith atlas (http://brain-development.org) with modified posterior fossa regions, combined regions of interest (ROIs; including aggregated regions for frontal, parietal, occipital, and temporal lobes, cingulate, and cerebellum) were spatially normalized to the T1-weighted MRI of each participant using Advanced Normalization Tools (ANTs) software (Avants et al., 2008). Further details of how PET images were subsequently processed are outlined in section 2.3.2.2.

A second MRI scan was acquired on a 3 Tesla Siemens MAGNETOM Prisma scanner, either on the same day as the PET scan or on a different day depending on patient tolerability. Sequences are outlined in Table 2-2 and were specifically used for the analysis in chapter 4 (where postsynaptic dendritic complexity was estimated using the Neurite Orientation Dispersion sequence). Other sequences were also acquired as part of this scan including diffusion tensor imaging (DTI), arterial spin labelling (ASL), resting-state functional MRI, T2-weighted sequences, as well as fluid attenuated inversion recovery (FLAIR), but were not used for the analyses in this thesis.

Modality	**Key parameters**
T1 - MPRAGE	Sagittal slices, TE=2.93 msec, TR=2s, flip angle 8°, slice thickness = 1.1 mm, resolution 1.1 mm^3 isotropic, 208 slices.
Neurite Orientation Dispersion Imaging - NODDI	TE = 75.6 msec, TR = 2.4s, slice thickness = 1.75mm, 104 slices, bvals = 300, 1000, 2000.

Table 2-2. Baseline High Resolution MRI protocol.
Siemens MAGNETOM Prisma scanner. TR = repetition time; TE= echo time.

2.3.2.2 Positron Emission Tomography imaging

All participants underwent synaptic imaging with [^{11}C]UCB-J PET (radiation exposure = 1.7 mSv), tau imaging with [^{18}F]AV-1451 PET (radiation exposure = 6.3mSv), with the CBS cohort also completing amyloid imaging with [^{11}C]PiB PET (radiation exposure = 2.9 mSv). All radioligands were prepared at the WBIC, with high radiochemical purity (>95%), and specific activity of >150 MBq, at the end of synthesis.

[^{11}C]UCB-J PET

Dynamic PET data acquisition was performed on a GE SIGNA PET/MR for 90 minutes starting immediately after [^{11}C]UCB-J injection with attenuation correction including the use of a multi-subject atlas. Each PET emission image series was aligned using SPM12 (www.fil.ion.ucl.ac.uk/spm/software/spm12/) then rigidly registered to the T1-weighted MRI acquired during PET data acquisition. Regional time-activity curves were extracted following the application of geometric transfer matrix (GTM) partial volume correction (PVC, (Rousset et al., 1998)) to each of the dynamic PET images. Regions of interest (ROIs) were multiplied by a binary grey matter mask (>50% on the SPM12 grey matter probability map smoothed to PET spatial resolution), with the exception of the pallidum, substantia nigra, pons and medulla because masking eliminated the ROI for some or all of the subjects. Multiple background grey matter, white matter and cerebrospinal fluid (CSF) regions were also defined to provide whole brain coverage for GTM PVC. The mean grey matter/(grey matter + white matter) fraction in the masked ROIs was 0.97 ± 0.03, 0.96 ± 0.03 and 0.96 ± 0.03 for the control, CBD and PSP groups, respectively, illustrating the predominance of grey matter in the masked ROIs. To assess the impact of PVC, time-activity curves were also extracted from the same ROIs without the application of GTM PVC.

To quantify synaptic density, [^{11}C]UCB-J non-displaceable binding potential (BP_{ND}) was determined, both regionally and at the voxel level, using a basis function implementation of the simplified reference tissue model (Wu & Carson, 2002), with the reference tissue defined in the centrum semiovale (Koole et al., 2019; Rossano et al., 2019). The volume-weighted average of the GTM PVC BP_{ND} values in the masked ROIs was used as a global BP_{ND} metric.

[^{18}F]AV-1451 PET

As for [^{11}C]UCB-J PET dynamic PET data acquisition here was also performed on a GE SIGNA PET/MR for 90 minutes starting immediately after [^{18}F]AV-1451 injection with attenuation correction including the use of a multi-subject atlas – preprocessing followed the steps outlined in Passamonti et al. (2017). Each PET emission image series was aligned using SPM12 to correct for patient motion during data acquisition

(www.fil.ion.ucl.ac.uk/spm/software/spm8). The mean aligned PET images were rigidly registered to the T1-weighted images acquired during PET data acquisition. This allowed extraction of values from both the Hammers atlas regions of interest (ROIs) and those in a reference tissue defined in the inferior cerebellar grey matter (GM) using a 90% GM threshold on the GM probability map produced by SPM8 smoothed to PET resolution. Regional time-activity curves were extracted following the application of geometric transfer matrix (GTM) partial volume correction (PVC, (Rousset et al., 1998)) to each of the dynamic PET images. Regions of interest (ROIs) were multiplied by a binary grey matter mask (>50% on the SPM12 grey matter probability map smoothed to PET spatial resolution), with the exception of the pallidum, substantia nigra, pons and medulla because masking eliminated the ROI for some or all of the subjects. Multiple background grey matter, white matter and cerebrospinal fluid (CSF) regions were also defined to provide whole brain coverage for GTM PVC. [^{18}F]AV-1451 non-displaceable binding potential (BP_{ND}) was determined for each ROI using a basis function implementation of the simplified reference tissue model (Gunn et al., 1997). To assess the impact of PVC, time-activity curves were also extracted from the same ROIs without the application of GTM PVC.

[^{11}C]PiB PET

Amyloid PET imaging was carried out on a GE SIGNA PET/MR 50-70 minutes post injection of Pittsburgh Compound B ([^{11}C]PiB), in the CBS cohort only. Cortical standardised uptake value ratio (SUVR), was determined using the Centiloid Project methodology, with the whole cerebellum as the reference tissue (Klunk et al., 2015). Only those CBS participants with a negative amyloid status as characterised by a cortical [^{11}C]PiB SUVR less than 1.21 (obtained by converting the Centiloid cut-off of 19 to SUVR using the Centiloid-to-SUVR transformation) (Jack et al., 2017a), were included in the subsequent analyses, with the aim of excluding patients with CBS due to Alzheimer's disease. This amyloid-negative group is interpreted as having CBD, although I acknowledge that other pathologies are possible.

The total numbers of participants completing each arm of the study are outlined in Table 2-3 which reflects total recruitment numbers at the time of writing this thesis; the number

of participants forming the basis of the analysis in each chapter varies depending on the availability of data for each individual: for example, in chapter 4 not all participants with a NODDI sequence on the PRISMA scanner, had completed their [^{11}C]UCB-J scan. Likewise in chapter 5, not all patients with an [^{18}F]AV-1451 PET had completed their [^{11}C]UCB-J scan at the time of thesis write-up. The sample size for each analysis is therefore clarified in each respective chapter.

	Neuropsychological assessment	**Prisma MRI**	**[^{18}F]AV-1451**	**[^{11}C]UCB-J**
Control	27	27	22	20
PSP	25	25	25	23
CBS **-All** **-Amyloid negative on [^{11}C]PiB PET**	 25 14	 25 14	 24 14	 22 12

Table 2-3. Total number of participants completing each arm of the study.
PSP=Progressive Supranuclear Palsy - Richardson's Syndrome; CBS = Corticobasal Syndrome.

2.4 ANALYSIS

2.4.1 POWER CALCULATION

Given the novelty of this study, power could not be calculated on available pilot data. I therefore based the power calculations on our recent experience and data of [^{18}F]AV-1451 PET imaging in "NeuroInflaMation Research Of Dementia (NIMROD)" study in PSP and frontotemporal lobe dementia (Bevan-Jones et al., 2020; Bevan-Jones et al., 2018a; Bevan-Jones et al., 2018b; Passamonti et al., 2017), and extensive studies of other PET ligands in dementia groups. A sample size of 24 per group provides >85% power to detect medium to large effects sizes, d=0.8, for $\alpha<0.05$, and >85% power to detect correlations of moderate strength $|\rho|>0.5$. Recruiting 30 participants per group accommodates 10% technical and synthesis failure and 10% participant attrition from withdrawal or co-morbidity. Power calculations used the GPower software.

2.4.2 STATISTICAL ANALYSIS

Analysis primarily adopts regions of interest approach using parametric statistics from general linear models of group differences (patient versus control), and correlations within the patient group against clinical severity. Where multimodal imaging is involved, linear mixed effect models have been utilised. Where data distributions indicated the need for

non-parametric tests, non-parametric analogues, and permutations tests have been used. All statistical analysis were performed in the statistical package R (version 3.6.2). Specific details are provided in each respective experimental chapter.

3 SYNAPTIC LOSS IN PRIMARY TAUOPATHIES REVEALED BY [^{11}C]UCB-J POSITRON EMISSION TOMOGRAPHY

A manuscript related to this chapter has been published as:

Holland N, Jones PS, Savulich G, et al. Synaptic loss in the primary tauopathies, revealed by [^{11}C]UCB-J Positron Emission Tomography. *Mov Disord*. 2020;35(10):1834-1842. doi:10.1002/mds.28188.

The original data, on which the analyses in this chapter are based, were collected by myself, Dr George Savulich and Mrs Julie Wiggins. Patients were scanned and assessed as part of the Synaptic Evaluation in NeuroDegeneration Research (SENDeR) study which was conceived by Professor James Rowe and Professor John O'Brien. The PET data were pre-processed by Dr Tim Fryer and Dr Young Hong. I designed the analysis strategy, performed the analyses, constructed the figures, and wrote the text.

3 | Synaptic loss in primary tauopathies revealed by [^{11}C]UCB-J Positron Emission Tomography.

Summary

Synaptic loss is a prominent and early feature of many neurodegenerative diseases. In this chapter I test the hypothesis that synaptic density is reduced in the primary tauopathies of progressive supranuclear palsy (PSP-Richardson's syndrome) and amyloid–negative corticobasal syndrome (CBS). Thirty eight participants (9 CBD, 14 PSP, and 15 age-/sex-/education-matched controls) underwent positron emission tomography (PET) with the radioligand [^{11}C]UCB-J, which binds to synaptic vesicle glycoprotein 2A (SV2A), a marker of synaptic density. Participants also had 3T magnetic resonance imaging and clinical and neuropsychological assessment. All nine CBD patients had negative amyloid biomarkers determined by [^{11}C]PiB PET. Patients with PSP-Richardson's syndrome and CBD were impaired in executive, memory and visuospatial tasks. [^{11}C]UCB-J binding was reduced across frontal, temporal, parietal, and occipital lobes, cingulate, hippocampus, insula, amygdala and subcortical structures in both PSP and CBD patients compared to controls ($p<0.01$), with median reductions of up to 50%, consistent with post mortem data. Reductions of 20-30% were widespread even in the areas of the brain with minimal atrophy. There was a negative correlation between global [^{11}C]UCB-J binding and the PSP and CBD rating scales ($R= -0.61$ $p<0.002$, $R= -0.72$ $p<0.001$, respectively), and a positive correlation with the revised Addenbrookes Cognitive Examination ($R=0.52$, $p=0.01$). I confirm severe synaptic loss in PSP and CBD in proportion to disease severity, providing critical insight into the pathophysiology of primary degenerative tauopathies.

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3.1 INTRODUCTION

Both PSP and CBD are associated with cortical and subcortical atrophy on magnetic resonance imaging (MRI) (Jabbari et al., 2019) and changes in neurophysiology and connectivity measured by magnetoencephalography (Sami et al., 2018) and functional MRI (reviewed in Filippi et al. (2019)), respectively. However, functional changes are also seen in the areas of the brain that are minimally atrophic, as reviewed in chapter 1.1.6. In this chapter I test the hypothesis that the neurophysiological and functional impairments in PSP and CBD are at least in part a consequence of synaptic loss. For example, at post mortem there is approximately 50% loss of cortical synapses in PSP and CBD (Bigio et al., 2001; Lipton et al., 2001), and *in vivo* there is limited evidence of a ~20% loss of postsynaptic $GABA_A$ receptors as shown with [^{11}C]flumazenil positron emission tomography (PET) (J. D. Andersson et al., 2019; Foster et al., 2000). Indeed abnormal physiology in pathways involved in presynaptic function have been identified from transcriptomic studies in patients with mutations in the microtubule-associated protein tau (MAPT) gene (Jiang et al., 2018). Transgenic models of tauopathies (e.g. rTg4510) confirm a synaptotoxic effect of oligomeric tau, before cell death (Kaniyappan et al., 2017; Menkes-Caspi et al., 2015). Moreover, in other neurodegenerative dementias, such as Alzheimer's disease, synaptic loss correlates better with cognitive dysfunction than atrophy (Terry et al., 1991). To date however, there have been no direct *in vivo* assessment of synaptic density in PSP and CBD.

Before outlining the methodology, I will review the normal physiological role of the synapse, the evidence suggesting a central role in cognition and the techniques used for measuring synapses *in vivo.*

3.1.1 THE SYNAPSE

3.1.1.1 The physiological role, and the structure of the synapse

Synapses are fundamental components of neurons and allow an organised flux of information in the brain (Alberts et al., 1997). The emergence, diversification, and specialisation of synapses played a critical role in the evolution of higher brain functions and cognition in vertebrates (reviewed in Ryan and Grant (2009)). There are 100 trillion interconnecting synapses (Azevedo et al., 2009), each comprising of the pre- and postsynaptic membranes (separated by ~20 nm) and the synaptic cleft (Figure 3-1) (Stewart

et al., 2014). The efficiency of synaptic transmission and therefore intact cognition is dependent on the orchestrated activity and intact components of the pre- and postsynaptic membranes. Key to the process of neurotransmission is the release of neurotransmitter molecules from synaptic vesicles (residing in the presynaptic membrane) in response to an action potential induced rise in intracellular calcium. The presynaptic compartment contains the key molecules for formation, storage and release of neurotransmitter-containing vesicles (Südhof, 2013). Once released into the synaptic cleft, neurotransmitters bind to the postsynaptic membrane (also known as dendritic spines) and transmit signals through activating either the excitatory (glutamatergic transmission) or inhibitory pathways (GABAergic transmission) (Chang-Lu Tao et al., 2018; Hering & Sheng, 2001) . Fast, point-to-point synaptic transmission is enabled by the temporal and spatial restriction of neurotransmitter release and reception, and by the precise alignment of pre- and postsynaptic structures at the synaptic junction. The presynaptic membrane almost always belongs to a neuron, however although the postsynaptic membrane is mostly neuronal, it can also be part of non-neuronal oligodendrocyte precursor cells suggesting a key role for neuroinflammatory cells in regulating synaptic transmission (Südhof, 2004).

Synapses can be excitatory or inhibitory in nature, with slightly different structural morphology of the postsynaptic compartment. Excitatory synapses have an asymmetrical synaptic junction with a prominent postsynaptic density, and synaptic vesicles of spheroid character, whereas inhibitory synapses are typically GABA-ergic, have a synaptic junction that is symmetrical in form, do not contain a prominent postsynaptic density, and possess ellipsoidal synaptic vesicles (Stewart et al., 2014).

In humans, most synapses are formed during pre- and postnatal development, with approximately 50% pruned in the first 2 decades of life, with prefrontal cortical synaptic pruning continuing well into the 3rd decade (Stewart et al., 2014). Beyond this, synaptic numbers remain stable with a balance between some pruning and new synaptic formation, with a mild decline beyond the age of 80 (Peter R., 1979).

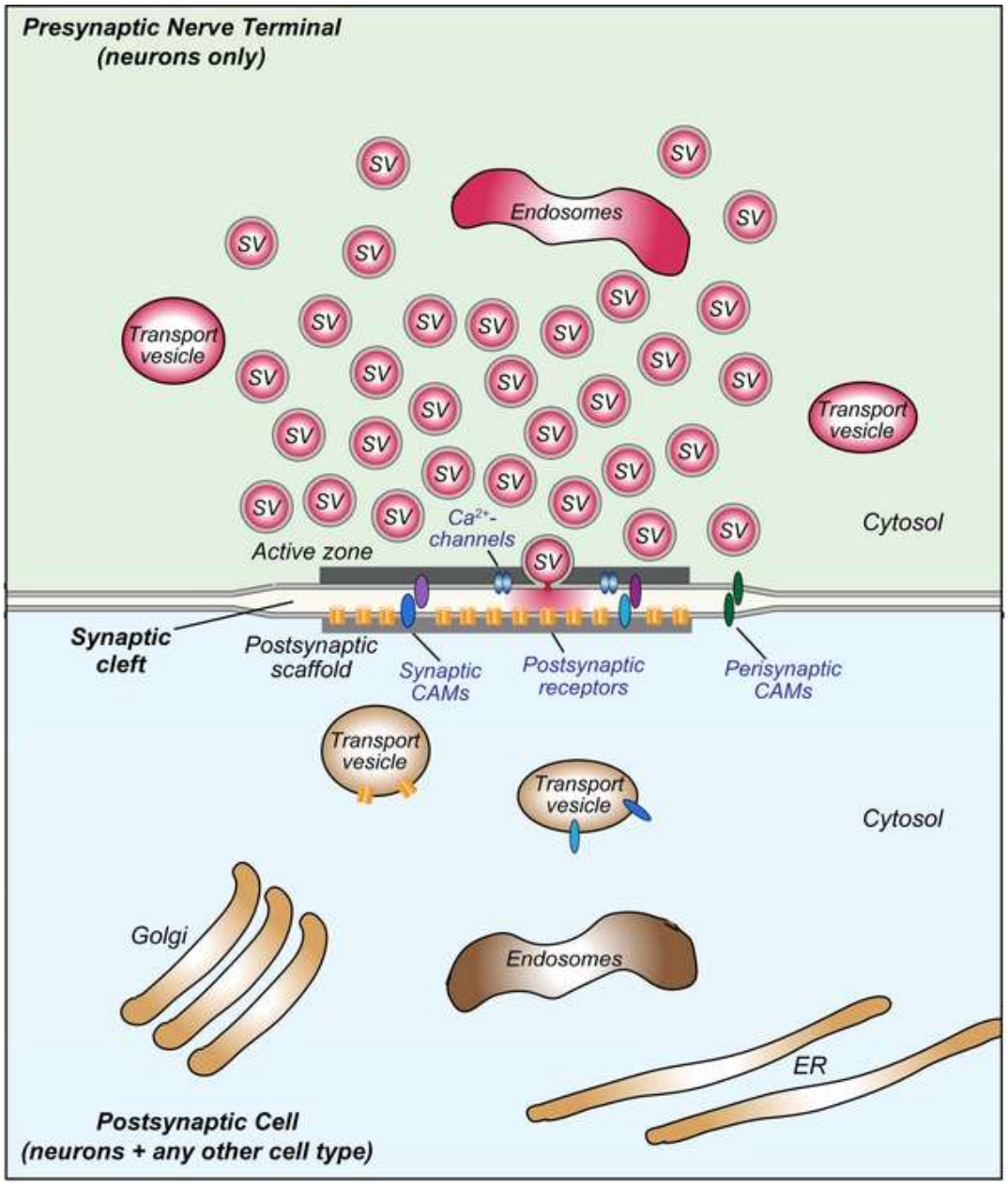

Figure 3-1. Schematic diagram of the components of a central synapse.
Schematic drawing of a synaptic junction with a cluster of synaptic vesicles (SV) on the presynaptic side and neurotransmitter receptors on the postsynaptic side. Pre- and postsynaptic transport vesicles for receptors, active zone components, and trans-synaptic cell-adhesion molecules (CAMs) are indicated, as well as presynaptic endosomes and postsynaptic organelles (Golgi apparatus, endosomes, and endoplasmic reticulum [ER]). In the brain, the synaptic cleft is usually wider than the surrounding interstitial space. All synapses contain similar presynaptic components independent of type, although the specific isoforms of various proteins (synaptotagmins, neurotransmitter transporters, Ca^{2+} channels, etc.) vary. In contrast, postsynaptic components of excitatory and inhibitory synapses exhibit no homology, neither at the level of receptors nor in the postsynaptic scaffolding proteins. Additionally, presynaptic specialisations are formed exclusively by neurons, but postsynaptic specialisations can likely be formed by any cell in the body. Figure reproduced from Sudhof 2018 with permission from Elsevier.

3.1.1.2 Synaptic vesicle and their membrane proteins

Key to synaptic transmission are synaptic vesicles which are packed within the presynaptic terminal of a synapse. There are several hundred to thousands of vesicles, each covered with numerous synaptic vesicle proteins on their membrane, containing neurotransmitters. In response to an action potential and calcium-dependent depolarisation of a presynaptic membrane, synaptic vesicles are primed, ready to fuse with the presynaptic terminal for neurotransmitter release. Synaptic vesicle distribution therefore appears to be related to functional activity with redistribution of vesicles in the synaptic bouton toward the synaptic active zone only occurring in response to an action potential. This mechanism underlies the phenomenon of long-term pentation and is the basis for learning and memory formation. The process of long-term potentiation involves a change in the efficacy of synaptic transmission, through changes in the morphology of synapses – this manifests as structural plasticity secondary to strengthening and growth of existing synapses and/or development of new synapses (Stewart et al., 2014).

Efficient synaptic transmission depends on three sets of synaptic vesicles that have been identified based on location within the presynaptic compartment: those that are primed and ready to be fused with the presynaptic membrane (0.5-5% of all vesicles); the recycling pool (5-20% of all vesicles) which form part of the exocytosis-endocytosis cycle under high frequency physiological conditions, and used repeatedly during sustained activity; the reserve or resting pool (80-90% of all vesicles) which are only used under intense stimulation or when the recycling pool is depleted (Südhof, 2004). The mobilisation, priming, and docking of vesicles at the active zone, and subsequent exo- and endocytosis of synaptic vesicles are dependent on key proteins within the synaptic vesicle membrane as shown in Figure 3-2.

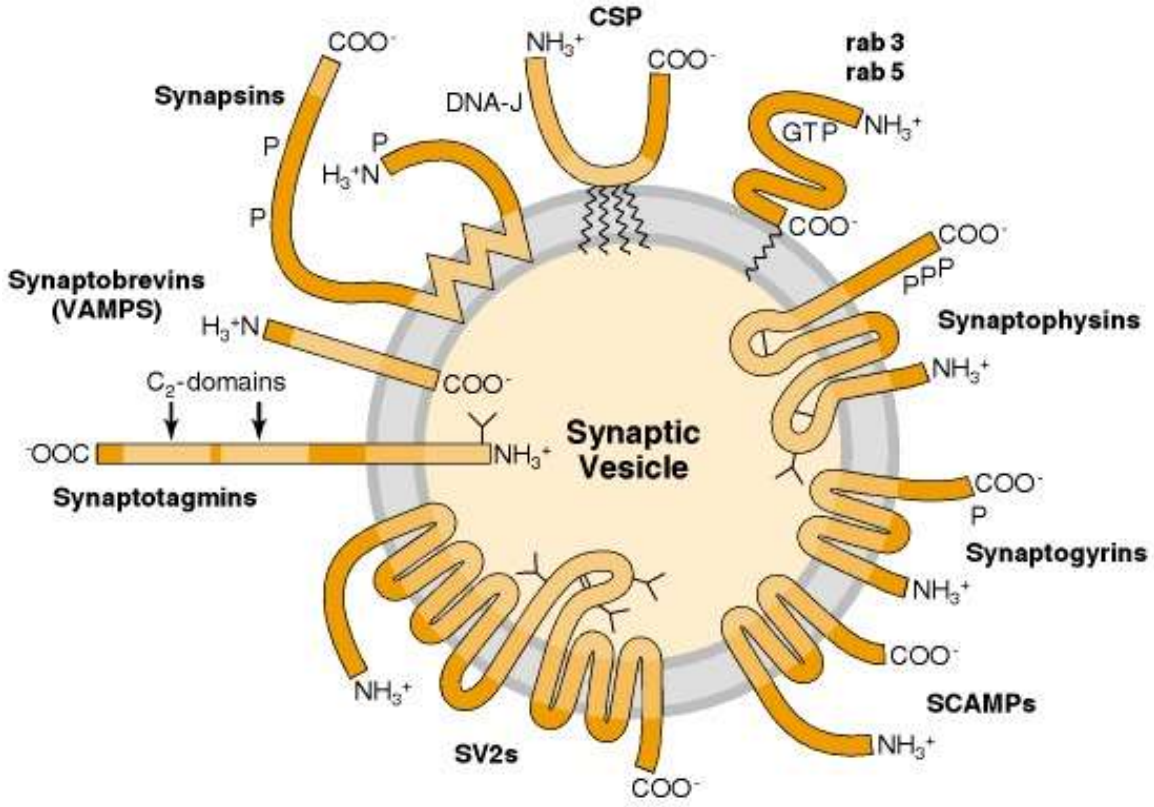

Figure 3-2. Synaptic Vesicle Proteins are key for neurotransmission.
Schematic diagram of the structure of presynaptic vesicle proteins; the specific proteins shown here are key in the synaptic vesicle cycle and trafficking. SCAMPS: secretory membrane carrier proteins; VAMPs: vesicle-associated membrane protein. CSP: cysteine string proteins. SVs: synaptic vesicle proteins. Figure reproduced from Südhof 1999, with permission from Nature publishing.

3.1.2 THE ROLE OF SYNAPSES IN COGNITION AND BEHAVIOUR

The concept of synaptic involvement in learning and memory was first postulated by Hebb stating that changes underlying learning and memory occur in three stages, the first including synaptic plasticity (Hebb, 1949):

"*When an axon of cell A is near enough to excite a cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased."*

Since the above postulation, many different methods have been devised to study synapses in the context of memory formation. These include post mortem studies of synaptic vesicle proteins, most commonly synaptophysin, the expression of which is considered a proxy for synaptic density (Hamos et al., 1989; Masliah et al., 1990). Alternative methods include *in*

vitro synaptic assessments in transgenic or knock-out mouse models. For example, knock-out mouse models lacking the presynaptic protein synaptotagmin-4 (key for neurotransmitter release), exhibit difficulties with hippocampal-dependent memory, contextual fear conditioning and the social transmission of food preference (Ferguson et al., 2000); synapsin-II knock-out mice show behavioural difficulties such as prepulse inhibition, decreased social behaviour and locomotor hyperactivity (Dyck et al., 2009) – an observation that was later replicated by downregulating the same protein within the medial-frontal cortex (Dyck et al., 2011). One caveat to these experimental models for the study of synaptic function in learning and memory however, is the widespread effect knock-out models have on reducing specific synaptic proteins across whole brain, therefore hindering association with particular behavioural deficits. Additionally, given such knock-out mouse models do not survive to maturity, studying behaviour across the age-span becomes difficult. An alternative approach is therefore to study synaptic protein downregulation in specific parts of the brain by selective blockade – for example by using tetanus toxin. Using this approach, localised reductions in hippocampal synaptobrevin-2 has been associated with deficits in spatial learning and memory recall (Südhof, 2004). Acknowledging the key role of synapses in learning and memory, synaptic dysfunction has become a key focus of research in neurodegenerative diseases with prominent cognitive dysfunction and dementia, where for example in Alzheimer's disease reductions have been reported as a better correlate with functional outcomes than neuronal loss (Scheff et al., 2006; Terry et al., 1991).

3.1.3 MEASURING SYNAPSES *IN VIVO*

The first method of quantifying synapses in patients with dementia utilised electron microscopy, however this technique is limited by its focus on a very small section of tissue, vulnerability to tissue processing and the need for rapid tissue fixation. Antigen-specific immunochemical detection methods have subsequently been developed. Given that synaptophysin is the most abundant synaptic vesicle protein, synaptophysin immunohistochemistry has been widely used as a marker of synaptic density, where its density as well as that of other synaptic proteins are reduced in PSP and CBD (Bigio et al., 2001; Lipton et al., 2001), as well as in Alzheimer's disease (Sze et al., 1997). Using the latter technique to assess synaptic density in humans is dependent on brain tissue from

autopsy or surgical resection, therefore greatly limiting the use of this approach for early diagnosis and therapeutic monitoring.

In vivo assessment of synaptic density is possible using positron emission tomography (PET). Previously, the most widely used PET radioligand for the study of synaptic function was fluorodeoxyglucose (F18) positron emission tomography (FDG-PET). FDG-PET is a marker of glucose metabolism, and its uptake is therefore highly affected by synaptic density and activity. Using this method glucose hypometabolism is reported in the central region, the frontal and parietal association fibres, the putamen contralateral to the clinically affected side and the thalamus in CBS, and bilateral anterior cingulate and the midbrain in PSP (Amtage et al., 2014; Hellwig et al., 2012; Hellwig et al., 2015; Zhao et al., 2012). Glucose hypometabolism is however not only affected by neuronal synapses but also by the surrounding neuroinflammatory cells – synaptic loss can therefore be misrepresented given increased in FDG-PET due to neuroinflammation in PSP and CBS. An alternative *in vivo* assessment of synaptic density is through the use of synapse-specific PET radioligands, targeting the $GABA_A$ receptors ([^{11}C]flumazenil), dopamine receptors ([^{18}F]dopa and [^{11}C]raclopride), opioid receptors ([^{11}C]diprenorphine), and muscarinic acetylcholine receptors ([^{11}C]NMPB) (Niccolini & Politis, 2016) . Using dopamine and $GABA_A$ specific PET ligands in PSP and CBS, reductions in PET signal in hypometabolic cortical regions, and the basal ganglia, respectively have been observed. However, the interpretation of the signal from such receptor-specific scans is limited to the areas of the brain where these receptors are primarily located.

More recently advances in synaptic imaging has allowed direct visualisation of synapses by targeting the synaptic vesicle 2A (SV2A) protein. SV2A is ubiquitous to all synaptic vesicles within the human brain except for the trigeminal and facial nuclei (Mendoza-Torreblanca et al., 2013). This synaptic vesicle protein is an integral 12-transmembrane domain glycoprotein forming 3 isoforms within the synaptic vesicle glycoprotein family, including SV2A, B, and C, where the latter two isoforms have a more restricted distribution (Bajjalieh et al., 1994; Bajjalieh et al., 1993b). SV2A is present in both excitatory and inhibitory synapses, where it acts as a transporter and a gel matrix for the immobilisation and subsequent liberation of neurotransmitters; given its acidic N terminus, it is also postulated to be involved in calcium-dependent exocytosis (Südhof, 2004). SV2A plays a

key role in the pathogenesis of epilepsy, where knock-out mice manifest with severe seizures, making this protein a focus for anti-epileptic drug targets. One such drug is levetiracetam (Keppra) which directly binds SV2A (Lynch et al., 2004), giving the opportunity to use its chemical structure in the recent development of the synaptic vesicle 2A specific PET radioligand [^{11}C]UCB-J ((*R*)-1-((3-(methyl-^{11}C)pyridin-4-yl)methyl)-4-(3,4,5-trifluorophenyl)pyr-rolidin-2-one) (Finnema et al., 2016).

To date, [^{11}C]UCB-J has been widely used in Alzheimer's research where widespread reductions in SV2A binding have been reported in the medial temporal and neocortical brain regions in early disease, beyond the extent of grey matter volume loss. Furthermore, reductions in hippocampal SV2A binding were correlated with episodic memory loss and the clinical dementia rating scale (M. K. Chen et al., 2018; Mecca et al., 2020).

I therefore use [^{11}C]UCB-J PET to test the hypothesis that PSP and CBS reduce synaptic density, in proportion to disease severity. In this experimental chapter I include patients with the classic phenotype of PSP, PSP-Richardson's syndrome, which has a high clinicopathological correlation (Gazzina et al., 2019). I include patients with Corticobasal Syndrome (CBS) with probable underlying CBD. In order to do this, it is necessary to exclude the substantial minority of CBS caused by Alzheimer's disease pathology (Alexander et al., 2014). I therefore used amyloid imaging to distinguish those with CBS due to CBD, *versus* Alzheimer's disease; I refer to this group as the CBD cohort. I further assess correlations between regional [^{11}C]UCB-J binding potential, a metric of synaptic density, and disease severity, in terms of cognitive decline and global impairment on the PSP and CBD rating scales.

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3.2 METHODS

3.2.1 PARTICIPANTS AND STUDY DESIGN

Fourteen patients with PSP-Richardson's syndrome and fifteen patients with CBS were recruited from a tertiary specialist clinic for PSP/CBS at the Cambridge University Centre for Parkinson-Plus. Fifteen healthy volunteers were recruited from the UK National Institute for Health Research Join Dementia Research (JDR) register. Patients had either probable PSP–Richardson Syndrome (Höglinger et al., 2017), or both probable CBS and probable CBD (Armstrong et al., 2013). Healthy controls and patient volunteers were initially screened by telephone; exclusion criteria were as per Chapter 2 (2.1.3). Eligible participants were invited for a research visit where they underwent clinical and cognitive assessment including measures of disease severity (Table 1); these included a neurological examination by a clinician including the PSP and CBD rating scales, the Schwab and England Activities of Daily Living (SEADL) and Clinical Dementia Rating Scale (CDR). Cognitive testing included the revised Addenbrooke's Cognitive Examination (ACE-R), the Mini-mental State Examination (MMSE), and the INECO frontal assessment test. Patients' carers completed the revised Cambridge Behavioural Inventory (CBI).

All participants underwent simultaneous 3T MRI and [^{11}C]UCB-J PET. Patients with CBS also underwent amyloid PET imaging using Pittsburgh Compound B ([^{11}C]PiB) and cortical standardised uptake value ratio (SUVR; 50-70 minutes post injection; whole cerebellum reference tissue) was determined using the Centiloid Project methodology (Klunk et al., 2015). Only those with a negative amyloid status as characterised by a cortical [^{11}C]PiB SUVR less than 1.21 (obtained by converting the Centiloid cut-off of 19 to SUVR using the Centiloid-to-SUVR transformation) (Jack et al., 2017b), are included in the subsequent analysis, with the aim of excluding patients with CBS due to Alzheimer's disease. I interpret this amyloid-negative group as having CBD, although acknowledge that other pathologies are possible.

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3.2.2 NEUROIMAGING PRE-PROCESSING

[^{11}C]UCB-J was synthesised at the Radiopharmacy Unit, Wolfson Brain Imaging Centre, Cambridge University, using the methodology previously described (Milicevic Sephton et al., 2020) . Dynamic PET data acquisition was performed on a GE SIGNA PET/MR (GE Healthcare, Waukesha, USA) for 90 minutes starting immediately after [^{11}C]UCB-J injection (median injected activity: 351 ± 107 MBq, injected mass ≤ 10 μg), with attenuation correction including the use of a multi-subject atlas method (Burgos et al., 2014) and also improvements to the MRI brain coil component (Manavaki et al., 2019). Each emission image series was aligned using SPM12 (www.fil.ion.ucl.ac.uk/spm/software/spm12/) then 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 (subsequently interpolated to 1.0 x 1.0 mm); slice thickness 1.0 mm). Using a version of the Hammersmith atlas (http://brain-development.org) with modified posterior fossa regions, combined regions of interest (including aggregated regions for frontal, parietal, occipital, and temporal lobes; cingulate; and cerebellum) were spatially normalized to the T1-weighted MRI of each participant using Advanced Normalisation Tools (ANTs) software(Avants et al., 2008). Regional time-activity curves were extracted following the application of geometric transfer matrix (GTM) partial volume correction (PVC, (Rousset et al., 1998)) to each of the dynamic PET images. Regions of interest (ROIs) were multiplied by a binary grey matter mask (>50% on the SPM12 grey matter probability map smoothed to PET spatial resolution), with the exception of the pallidum, substantia nigra, pons and medulla because masking eliminated the ROI for some or all of the subjects. Multiple background grey matter, white matter and cerebrospinal fluid (CSF) regions were also defined to provide whole brain coverage for GTM PVC. The mean grey matter/(grey matter + white matter) fraction in the masked ROIs was 0.97 ± 0.03, 0.96 ± 0.03 and 0.96 ± 0.03 for the control, CBD and PSP groups, respectively, illustrating the predominance of grey matter in the masked ROIs. To assess the impact of PVC, time-activity curves were also extracted from the same ROIs without the application of GTM PVC.

To quantify SV2A density, [^{11}C]UCB-J non-displaceable binding potential (BP_{ND}) was determined, both regionally and at the voxel level, using a basis function implementation

of the simplified reference tissue model (Wu & Carson, 2002), with the reference tissue defined in the centrum semiovale (Koole et al., 2019; Rossano et al., 2019). The volume-weighted average of the GTM PVC BP_{ND} values in the masked ROIs was used as a global BP_{ND} metric. Group average BP_{ND} images (illustrated in Figure **3-3**) were obtained by spatially normalising each individual T1-weighted MRI (and thereby the co-registered BP_{ND} map) to MNI space, and then to the group template using ANTs.

3.2.3 STATISTICAL ANALYSIS

Statistical analyses used R (version 3.6.2), with analysis of covariance (ANCOVA) to compare regional [^{11}C]UCB-J BP_{ND} between the three groups (Control, CBD, PSP), with age as a covariate of no interest. Regions of interest were: frontal, temporal, parietal, occipital lobes; cingulate cortex, hippocampus, insula, amygdala, caudate nucleus, nucleus accumbens, putamen, pallidum, thalamus, cerebellum, substantia nigra, midbrain, pons, and medulla.

The relationships between [^{11}C]UCB-J BP_{ND}, disease severity (PSP and CBD Rating Scales) and cognition (revised Addenbrooke's Cognitive Examination) were tested through linear models of the patient data, with age as a covariate of no interest.

The primary analyses used BP_{ND} determined following GTM PVC, but all analyses were repeated using BP_{ND} without PVC yielding similar results.

3.3 RESULTS

3.3.1 CLINICAL SUMMARY AND DEMOGRAPHICS

Of the fifteen patients with CBS, six had a cortical [^{11}C]PiB SUVR more than 1.21 and were therefore excluded from further analysis in this chapter. The remaining groups (9 CBD, 14 PSP, 15 controls) were matched in age, sex and education (Table 3-1). We observed typical cognitive profiles, as summarised in Table 1: patients were impaired on memory, verbal fluency, language, and visuospatial domains of the ACE-R, and MMSE. There were high endorsements on the Cambridge Behavioural Inventory, and the Clinical Dementia Rating scale, with impairment of activities of daily living on the Schwab and England scale. Patients were not on any medications that are known to affect [^{11}C]UCB-J

binding. Four of our patients (1 PSP, 3 CBD) were on dopaminergic medication, and 9 on amantadine (3 PSP, 6 CBD).

	Control	CBD	PSP	F (p)
M:F	7:8	7:2	7:7	ns[a]
Age at [^{11}C]UCB-J PET in years	68 (7.45)	70.56 (8.23)	72.79 (7.74)	ns
Disease duration in years	NA	3.94 (2.2)	4.28 (2.57)	ns[b]
Education in years	13.69 (2.66)	12.78 (3.27)	12.77 (5.43)	ns
ACE-R total (max. 100)	96.47 (2.88)	81.56 (10.83)	80.57 (15.02)	9.61 (<.0004)
Attention_Orientation (max .18)	17.87 (0.35)	16.89 (1.05)	16.43 (2.06)	4.11 (0.02)
Memory (max .26)	24.53 (1.85)	20.67 (5.66)	21.43 (4.27)	3.50 (0.04)
Fluency (max .14)	12.80 (1.15)	8.22 (2.86)	6.43 (3.44)	22.81 (<.001)
Language (max .26)	25.53 (0.92)	22.44 (5.34)	23.43 (5.21)	ns
Visuospatial (max .16)	15.73 (0.59)	13.33 (2.55)	12.86 (3.98)	4.46 (0.02)
MMSE (max. 30)	29.27 (1.33)	26.44 (3.13)	27.00 (2.88)	4.78 (0.01)
PSPRS (max. 100)	0.13 (0.52)	26.78 (9.61)	29.21 (10.27)	ns[b]
CBDRS (max. 124)	0.20 (0.77)	29.12 (13.52)	36.80 (20.41)	ns[b]
INECO (max. 30)	26.00 (1.85)	14.6 0(8.47)	17.70 (4.74)	17.22 (<.001)
CDR sum of boxes (max. 32)	0.07 (0.26)	6.78 (4.71)	7.54 (6.55)	11.28 (<.001)
CBI (max. 180)	2.47 (4.81)	27.44 (13.5)	42.43 (38.13)	9.97 (<.001)
SEADL (max. 1)	0.99 (0.03)	0.56 (0.28)	0.60 (0.22)	10.54 (<.001)

Table 3-1. Demographics and neuropsychological profile for each participant cohort
The results are given as mean (standard deviation). CBD here refers to corticobasal syndrome with a negative amyloid biomarker from [^{11}C]PiB PET, and PSP refers to patients with PSP-Richardson's syndrome. The F-statistic and p-values are derived from ANOVA. ACE-R: revised Addenbrooke's Cognitive Examination, MMSE: Mini-mental State Examination, PSPRS: Progressive Supranuclear Palsy Rating Scale, CBDRS: CBD functional rating scale, INECO: frontal assessment tool, CDR: Clinical Dementia Rating Scale, CBI: revised Cambridge Behavioural Inventory, SEADL: Schwab and England Activities of Daily Living Scale. [a] chi-squared test. [b] ANOVA with PSP and CBD patients only. ns = non-significant at p<0.05; NA = non-applicable.

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3.3.2 WIDESPREAD REDUCTION IN CORTICAL AND SUBCORTICAL SYNAPTIC DENSITY

Compared to controls, in patients there was a significant global reduction in [^{11}C]UCB-J BP_{ND} (Figure **3-3**) across all major cortical and subcortical areas (p<0.05 FDR corrected for all regions of interest shown in Figure 3-3); regional BP_{ND} values for the three groups are reported in Table 3-2. BP_{ND} in PSP and CBD was 20-50% lower than controls (p<0.01),

with the most severe median reduction seen in the medulla, substantia nigra, pallidum, midbrain, pons and caudate nucleus in patients with PSP, and in the medulla, hippocampus, amygdala, caudate nucleus, insula and thalamus in patients with CBD. Post-hoc analysis revealed that the significant differences in BP_{ND} between patients and controls in the pallidum and substantia nigra were mainly driven by the PSP cohort. Using data without GTM PVC, the pattern of statistically significant differences in BP_{ND} for the reported regions in Table 2 remains, $p<0.001$.

3.3.3 SYNAPTIC LOSS OCCURS BEYOND THE EXTENT OF ATROPHY

The reduction in synaptic density was seen even in the areas of the brain that did not show significant grey matter atrophy. Figure 3-4A shows group differences in grey matter volume normalized against the mean of the control group. The significant areas of grey matter volume loss were in the caudate nucleus ($p=0.01$), and thalamus ($p=0.04$) in the CBD cohort, and in the frontal ($p<0.01$), temporal ($p=0.04$), parietal ($p<0.01$), occipital lobes ($p<0.01$), caudate nucleus ($p<0.001$), and the thalamus ($p<0.01$) in the PSP cohort. The reduction in [^{11}C]UCB-J BP_{ND} however, is more extensive and consistently significantly different across all major cortical and subcortical areas as shown in the normalised plot in Figure 3-4B (binding potentials were normalised against the mean binding potential of the control cohort for each region of interest).

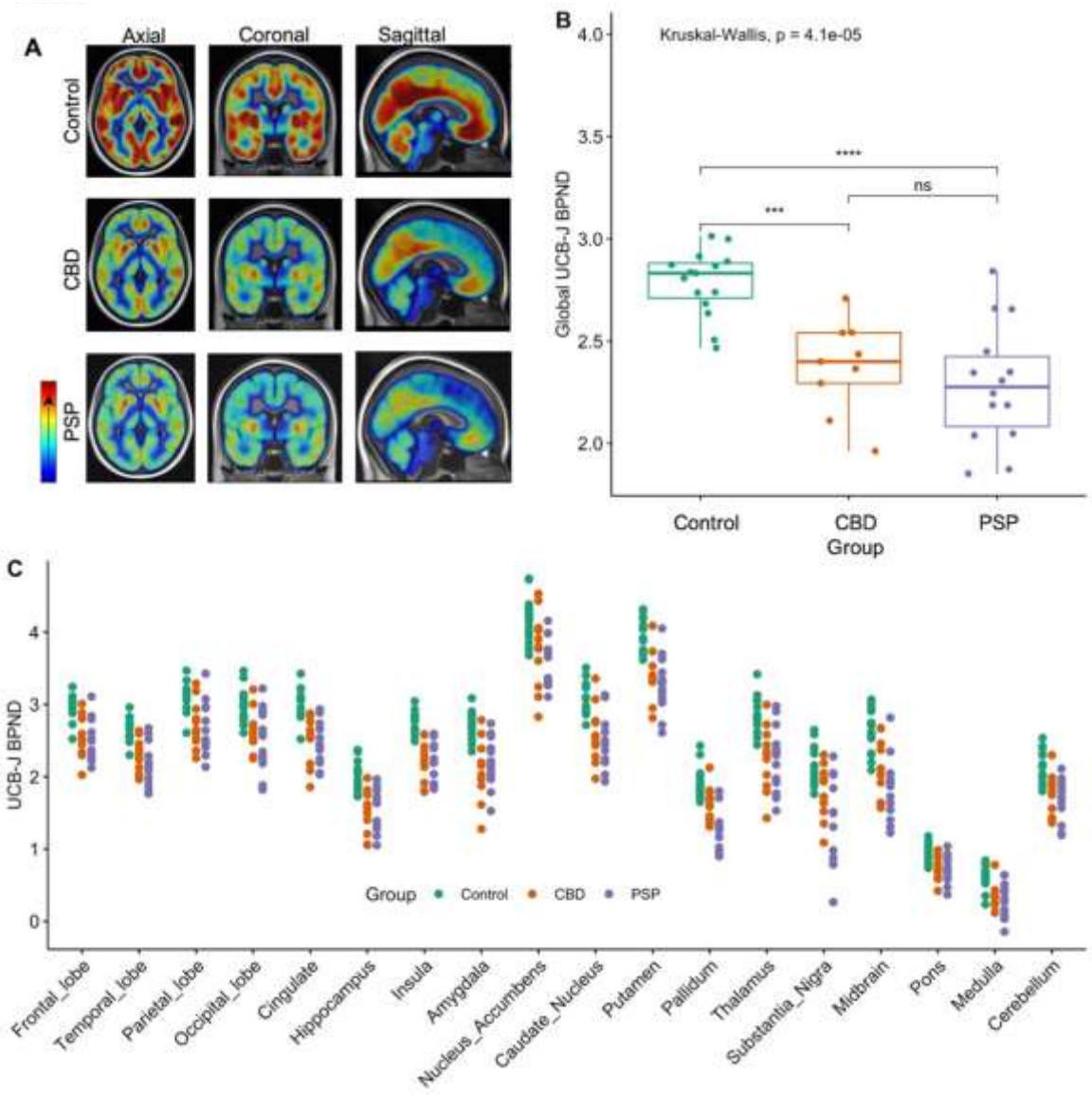

Figure 3-3. Widespread reductions in cortical and subcortical synaptic density in PSP and CBD.

(A) Mean [^{11}C]UCB-J BP_{ND} maps for control participants (top row), CBD (middle row), and PSP (bottom row); high and low BP_{ND} values are shown by red and blue areas, respectively. (B) Reduction in global [^{11}C]UCB-J BP_{ND} across patients compared to controls ($P<0.05$). (C) Individual regional GTM PVC [^{11}C]UCB-J BP_{ND} values for control, CBD and PSP participants, across major regions of interests. Binding potential values for patients differed significantly from controls in all the regions depicted ($P<0.05$, FDR corrected).

Region	Control	CBD	PSP	F (p)
Frontal Lobe	2.96 (0.17)	2.60 (0.29)	2.48 (0.28)	15.05 (<0.0001)
Temporal Lobe	2.68 (0.16)	2.30 (0.23)	2.17 (0.27)	19.34 (<0.0001)
Parietal Lobe	3.11 (0.19)	2.75 (0.32)	2.63 (0.36)	10.10 (<0.0003)
Occipital Lobe	2.98 (0.23)	2.66 (0.29)	2.48 (0.41)	8.80 (0.0008)
Cingulate	3.02 (0.21)	2.56 (0.26)	2.46 (0.28)	20.41 (<0.0001)
Insula	2.76 (0.15)	2.24 (0.26)	2.17 (0.27)	28.55 (<0.0001)
Amygdala	2.71 (0.20)	2.18 (0.34)	2.20 (0.33)	14.67 (<0.0001)
Nucleus Accumbens	4.18 (0.31)	3.85 (0.46)	3.54 (0.33)	11.28 (0.0002)
Hippocampus	2.00 (0.20)	1.57 (0.29)	1.57 (0.30)	12.37 (<0.0001)
Caudate Nucleus	3.12 (0.22)	2.59 (0.41)	2.48 (0.36)	15.59 (<0.0001)
Pallidum	1.90 (0.22)	1.65 (0.24)	1.27 (0.31)	20.69 (<0.0001)[a]
Putamen	3.99 (0.24)	3.43 (0.32)	3.28 (0.37)	19.94 (<0.0001)
Thalamus	2.86 (0.25)	2.29 (0.45)	2.25 (0.44)	11.23 (<0.0002)
Cerebellum	2.13 (0.22)	1.75 (0.30)	1.69 (0.28)	11.50 (0.0001)
Midbrain	2.61 (0.29)	2.16 (0.38)	1.83 (0.42)	16.61 (<0.0001)
Substantia Nigra	2.13 (0.28)	1.72 (0.34)	1.32 (0.59)	12.79 (<0.0001)[a]
Pons	0.93 (0.13)	0.75 (0.18)	0.71 (0.18)	7.69 (0.002)
Medulla	0.62 (0.16)	0.37 (0.17)	0.28 (0.24)	12.04 (<0.001)

Table 3-2. Mean (standard deviation) GTM PVC [^{11}C]UCB-J BP_{ND} values per group.
Values are shown for cortical and subcortical regions of interest (surviving false discovery rate correction over 18 regions). CBD here refers to corticobasal syndrome with a negative amyloid biomarker from [^{11}C]PiB PET, and PSP refers to patients with PSP-Richardson's syndrome. F-statistic and p-values derived from an ANCOVA across the three groups, with age as a covariate of no interest. [a]the significant difference here is driven by the PSP group only.

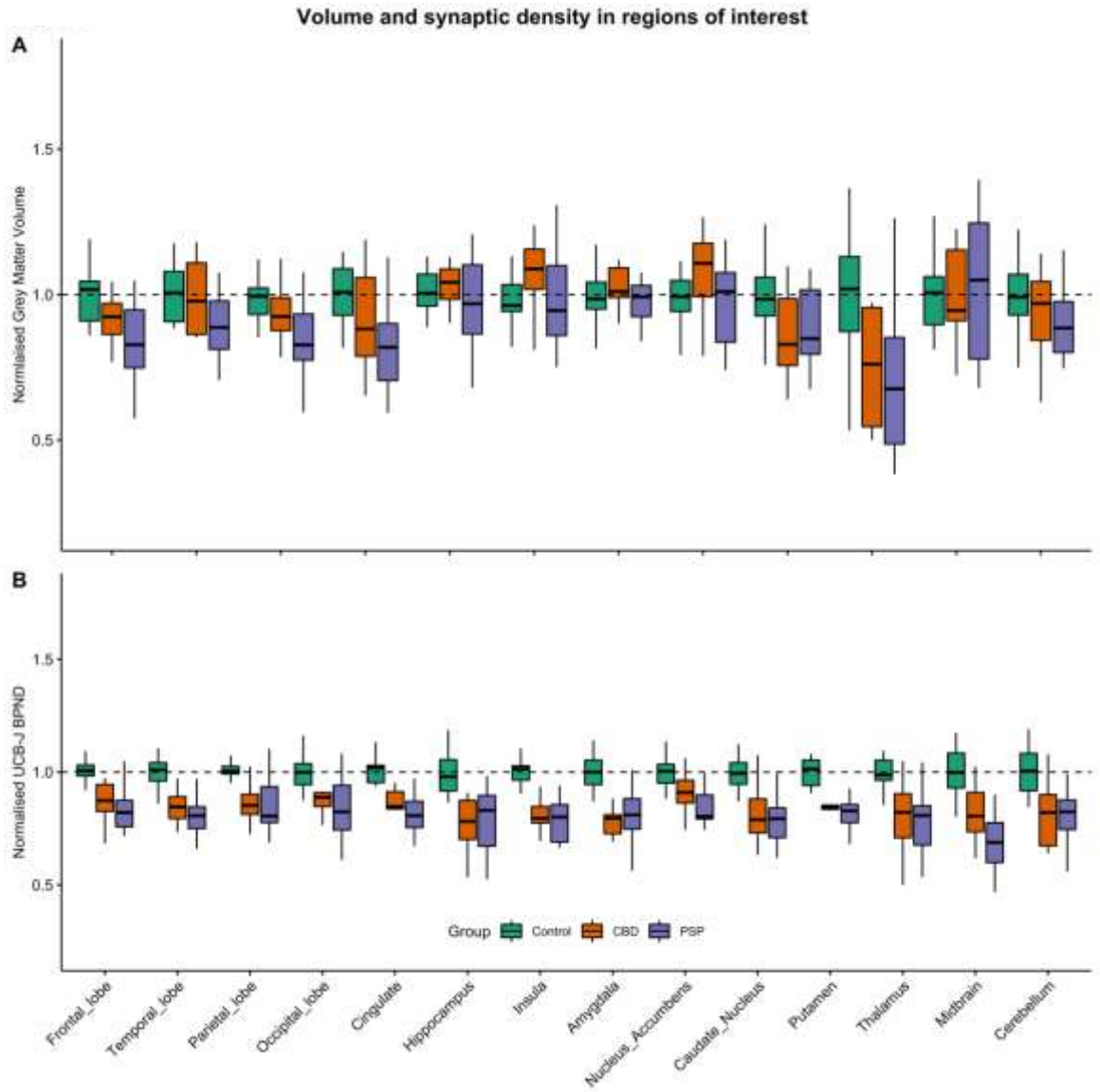

Figure 3-4. Normalised grey matter volume and [^{11}C]UCB-J BP_{ND} in regions of interest.
(A) Cortical and subcortical grey matter volumes, normalised against the corresponding volumes in controls, were significantly reduced in the caudate nucleus and thalamus in CBD; and in frontal, temporal, parietal and occipital lobes, as well as in the caudate nucleus, and thalamus in PSP, $P<0.05$. (B) Mean-centred [^{11}C]UCB-J BP_{ND} across cortical and subcortical regions of interest normalised against the corresponding BP_{ND} values in controls, demonstrating a median reduction of 20-50%.

3 | Synaptic loss in primary tauopathies revealed by [^{11}C]UCB-J Positron Emission Tomography.

3.3.4 SYNAPTIC LOSS CORRELATES WITH DISEASE SEVERITY AND GLOBAL COGNITION

Correlations between [^{11}C]UCB-J BP_{ND} and both global cognition and disease severity are given in Figure 3-5. A significant positive correlation was seen between global [^{11}C]UCB-J BP_{ND} and the revised Addenbrooke's Cognitive Examination total score (R=0.52, p=0.01) (Figure 3-5A). There was a significant negative correlation between global [^{11}C]UCB-J BP_{ND} and the PSP (R= -0.61, p<0.01), and CBD (R= -0.72, p<0.001) rating scales (Figure 3-5B&C).

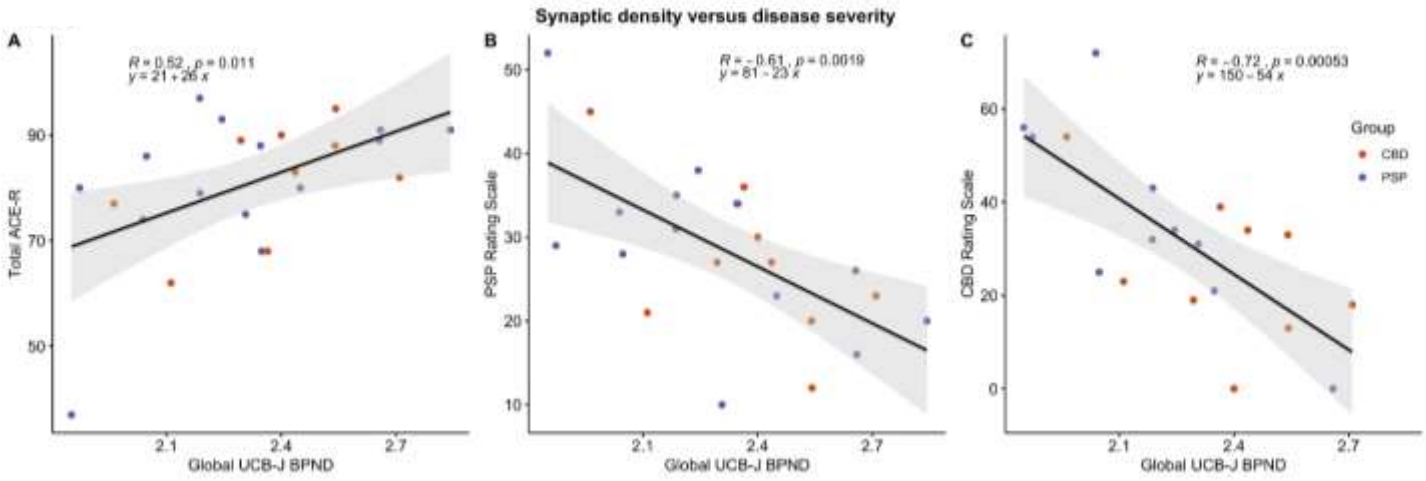

Figure 3-5. Synaptic loss correlates with disease severity and global cognition.
Correlations between global [^{11}C]UCB-J BP_{ND} and total ACE-R score (A), total PSP rating scale (B), and total CBD rating scale (C) for the two patient groups.

3.4 DISCUSSION

The principal result of this experimental chapter is a widespread reduction in synaptic density in PSP-Richardson's syndrome and amyloid-negative Corticobasal Syndrome (CBS) (which I define as Corticobasal Degeneration (CBD)). This accords with post mortem estimates of synaptic loss in PSP and CBD, using synaptophysin immunohistochemistry (Bigio et al., 2001; Lipton et al., 2001), imaging of neurite density in PSP (Mitchell et al., 2019) and morphological studies of cortical dendrites in the closely related condition of frontotemporal lobe dementia (I Ferrer et al., 1991). Indirect evidence of synaptic loss, from consequential reduction in metabolism, comes from [^{18}F]FDG PET changes in frontal, temporal and parietal lobes (Blin et al., 1992; Eidelberg et al., 1991; Foster et al., 1988; Juh et al., 2004). However, PET imaging with the ligand [^{11}C]UCB-J provides direct evidence *in vivo* of severe and extensive loss of cortical and subcortical synapses, including areas of the brain that are minimally atrophic (Josephs et al., 2008).

Progressive supranuclear palsy and corticobasal degeneration are progressive, with an average disease duration of five to eight years from symptom onset (Coyle-Gilchrist et al., 2016). In our clinically diagnosed CBD and PSP groups, the mean symptom duration at the time of PET was three and a half years, and our patients were likely to be approximately mid-way through their symptomatic disease course (not including a potentially long pre-symptomatic period). The median reduction of 20% (and maximal 50%) in [^{11}C]UCB-J binding observed *in vivo* compared to controls, is therefore in keeping with the predictions from post mortem data.

The synaptic loss observed in our study is widespread, extending beyond the regions that are arguably most associated with the diseases. In PSP, from post mortem studies, these include basal ganglia, thalamus, substantia nigra, premotor cortex, as well as the dentate nucleus. In CBD, areas associated with the disease include cortex, thalamus, basal ganglia and brainstem, without significant cerebellar involvement (Dickson et al., 2011; Kovacs et al., 2020). However, in our study the loss of synapses in PSP is global across the cortex, and not confined to the premotor and motor areas, and extends beyond the substantia nigra in the brainstem with pontine and medullary involvement. The loss of synapses in the cerebellum in PSP echoes pathological studies of tau distribution in this disease.

Interestingly, the cerebellum was also markedly abnormal in CBD; although cerebellar atrophy and tau accumulation are not typical associations of CBD. Cerebellar synaptic loss in CBD may therefore represent cerebellar diaschisis in response to widespread cortical pathology and loss of cortico-cerebellar projections; an alternative explanation is that a small minority of individuals in an amyloid-negative CBS cohort may have PSP as the underlying cause for their corticobasal syndrome, although this is unlikely to be sufficient to drive the group-wise effect.

Preclinical models of tauopathy suggest early synaptotoxicity with reduced plasticity and density (Menkes-Caspi et al., 2015), in response to soluble oligomeric tau aggregates (Kaniyappan et al., 2017) and inflammation (Rajendran & Paolicelli, 2018; Vogels et al., 2019). Recently in patients with Alzheimer's disease, using [^{11}C]UCB-J, [^{18}F]flortaucipir (PET radioligand targeting tau), and magenotoencephalography (used to assess cortical function *in vivo*), Coomans *et al* have demonstrated synaptic reductions with increasing tau load, both associated with reduced synaptic function on electrophysiology (Coomans et al., 2021). The toxicity associated with tau pathology leading to synapse loss is complex and involves direct and indirect pathways (reviewed in Spires-Jones and Hyman (2014)). Naturally occurring tau plays a role in synaptic function through modulating microtubule and axonal stability; disruptions to this machinery leads to prevention of the trafficking of essential components to synapses such as synaptic receptors and mitochondria (Hoover et al., 2010; Kopeikina et al., 2011). Indeed, over-expression of tau interferes with mitochondria transport (Stoothoff et al., 2009), and contributes to hyperexcitability of neurons and impaired calcium influx in transgenic mouse models (rTg4510) (Rocher et al., 2010).

The global nature of synaptic reduction suggests a more widespread pathology in the primary tauopathies of PSP and CBD beyond the areas that are histologically reported as harbouring a high tau burden such as the basal ganglia, thalamus, and brain stem (Dickson et al., 2011; Kovacs et al., 2020). This may in part be explained by the global damage caused by oligomers of tau which are not easily visible on tau PET imaging or histology. In support of this are biochemical studies that report tau accumulation in both grey and white matter by western blot in PSP but not necessarily by immunohistochemistry (Zhukareva et al., 2006). The pathological role of tau fibril is under debate, however toxic

tau oligomers in PSP (Gerson et al., 2014) are thought to play a crucial role in the pathogenesis of tauopathies through their gain of toxic function; this includes causing loss of membrane integrity (Flach et al., 2012), mitochondrial and synaptic dysfunction (Lasagna-Reeves et al., 2011b), and impairment of axonal transport, long-term potentiation, memory and cognition (Lasagna-Reeves et al., 2011b; Morfini et al., 2009; Ward et al., 2012).

In this experiment I observed a significant correlation between synaptic loss and disease severity in PSP and CBD. Synaptic loss correlates with cognitive impairment in another clinical tauopathy, Alzheimer's disease (Scheff et al., 2006; Terry et al., 1991), and preclinical models of this (Kandimalla et al., 2018; Lasagna-Reeves et al., 2011b; Walsh et al., 2002). The *in vivo* PET results in this chapter support the potential use of synaptic PET as a marker of disease and progression, but longitudinal data are required. Synaptic PET may support early stage clinical trials in PSP and CBS/CBD; it is encouraging in this latter respect that, in another neurodegenerative disease, [^{11}C]UCB-J PET is sensitive to progressive changes in synaptic density, for example in response to treatment with the synaptic modulator Saracatinib (Toyonaga et al., 2019).

This experimental chapter has a few limitations. The sample size is small, however it is adequately powered in view of the large effect sizes predicted. Nevertheless, subtler relationships with mild disease, progression or individual clinical features, or phenotypic variants of PSP and CBS, require larger studies. I acknowledge the potential for off-target binding, but preclinical data indicate very high correlations between UCB-J and synaptophysin, a marker of presynaptic vesicular density (Finnema et al., 2016; Finnema et al., 2018). The diagnoses used were clinical, without neuropathology, although the clinicopathological correlations of PSP-Richardson syndrome are very high, and in the absence of Alzheimer's disease, the clinicopathological correlation of CBS with a 4R-tauopathy (CBD or PSP) is also high. Binding potentials for SV2A radioligands such as [^{11}C]UCB-J can be confounded by the use of concurrent medication that may bind to SV2A. I did not enrol any individuals taking levetiracetam or any member of this family of drugs that are SV2A-specific ligands (Löscher et al., 2016). Previously reported studies using [^{11}C]UCB-J in disease have usually not commented on medications used by participants, however one study using this ligand in major depressive disorders reports exclusion of

participants on psychotropic medications in the 2 months preceding PET scanning (S. E. Holmes et al., 2019); whilst many of my PSP and CBD patients are on medications falling under the psychotropic umbrella, to my knowledge, none of these bind to SV2A.

Arterial blood sampling was not carried out in this study; I used reference tissue modelling to reduce the demand on the patient cohort. Reference tissue modelling of [^{11}C]UCB-J with the centrum semiovale as the reference tissue has been verified against arterial input function compartmental modelling in healthy controls (Koole et al., 2019; Rossano et al., 2019) and in Alzheimer's disease (M. K. Chen et al., 2018; Mecca et al., 2020). To assess the validity of the centrum semiovale in our cohort, we determined the mean total distribution volume (V_T) for each of our subject groups using standard arterial input function data from the literature (Finnema et al., 2018; Mansur et al., 2020); this approach assumed that the standard input function was equally valid for all groups. This analysis indicated a small positive bias in centrum semiovale V_T for CBD (5%) and less so PSP (2%) relative to that in controls, which would lead to a commensurate reduction in BP_{ND} under the assumption that the non-displaceable distribution volume (V_{ND}) in the target ROIs remains invariant. These biases cannot, however, explain the much greater BP_{ND} reductions seen for CBD and PSP, which is especially true for PSP. Indeed, scaling BP_{ND} in the CBD and PSP cohorts to account for the above biases in centrum semiovale V_T, produced a similar pattern of significant global reduction in BP_{ND} for patients compared to controls, except that the significant differences in the midbrain, pons, substantia nigra, pallidum, and occipital lobe were primarily driven by the PSP cohort in the post-hoc analysis.

The therapeutic challenge in tauopathies is partly due to the complex nature of the underlying pathology. Early stage trials will require early accurate diagnosis, although diagnosis is typically made 3 years after symptom onset (Coyle-Gilchrist et al., 2016; Mamarabadi et al., 2018). It is unlikely that synaptic PET could provide pre-symptomatic diagnosis in rare conditions, but it is a promising tool to characterise pathogenetic mechanisms, monitor progression and assess response to experimental medicines (Cai et al., 2019).

4 IN VIVO COUPLING OF DENDRITIC COMPLEXITY WITH PRESYNAPTIC DENSITY IN PRIMARY TAUOPATHIES

A manuscript related to this chapter has been published as:

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The original data, on which the analyses in this chapter are based, were collected by myself, Dr George Savulich and Mrs Julie Wiggins. Patients were scanned and assessed as part of the Synaptic Evaluation in NeuroDegeneration Research (SENDeR) study which was conceived by Professor James Rowe and Professor John O'Brien. The PET data were pre-processed by Dr Tim Fryer and Dr Young Hong, and the MRI Neurite Orientation and Dispersion Imaging by Dr Elijah Mak. Together with Dr Mak, I designed the analysis strategy, performed the analyses, constructed the figures, and wrote the text.

4 | In vivo coupling of dendritic complexity with presynaptic density in primary tauopathies

Summary

Understanding the cellular underpinnings of neurodegeneration remains a challenge. Loss of presynaptic density and postsynaptic dendritic complexity are characteristic and can be quantified *in vivo*, with [^{11}C]UCB-J PET and Orientation Dispersion Imaging (ODI, an MRI-based method), respectively. In this chapter I assess how the pre- and postsynaptic compartments are correlated *in vivo* in the 4-repeat tauopathies of Progressive Supranuclear Palsy and amyloid-negative Corticobasal Syndrome (CBS) as disease models of neurodegeneration. Patients with PSP (n= 24), and CBD (n= 15; amyloid negative as determined by [^{11}C]PiB PET) were recruited from a tertiary PSP/CBS clinic; 27 age- and gender-matched controls were recruited from the Join Dementia Research Platform. All participants underwent a 3T MRI with Neurite Orientation Dispersion Imaging (NODDI), [^{11}C]UCB-J PET, as well as neuropsychological testing. Patients with PSP and CBD had widespread reductions in cortical ODI, and [^{11}C]UCB-J non-displaceable binding potential (BP_{ND}) even when corrected for level of cortical atrophy, compared to healthy controls. Furthermore, presynaptic density as indexed by [^{11}C]UCB-J BP_{ND}, is tightly positively correlated with postsynaptic dendritic complexity, as indexed by ODI, across a wide range of cortical regions including the frontal and temporal cortices, as well as subcortical areas including the basal ganglia and brainstem (FDR corrected $p < 0.05$). This correlation was seen in the patient cohort only. Both measures, and the correlations between them, provide new insights into the pathophysiology of neurodegenerative diseases, and allow for a safe and widely available MRI platform to be used in future studies of synaptic health in neurodegeneration.

4.1 INTRODUCTION

Abnormal dendritic morphology, and synaptic pathology are increasingly recognised as hallmarks of neurodegeneration, prior to cell death and atrophy, and correlate closely with cognitive dysfunction (Clare et al., 2010; DeKosky & Scheff, 1990; Dorostkar et al., 2015; Terry et al., 1991). However, quantification of synaptic pathology *in vivo* has until recently posed a challenge. In the previous chapter, using the novel PET radioligand [^{11}C]UCB-J, I confirmed widespread loss of presynaptic density. Whether this pathogenic mechanism is associated with changes within the postsynaptic compartment, in primary tauopathies, has not been assessed *in vivo*.

4.1.1 POSTSYNAPTIC COMPARTMENT AND ITS PHYSIOLOGICAL ROLE

The postsynaptic compartment comprises of the dendritic spines of the postsynaptic neuron. Dendritic spines are numerous in numbers; for example, the frontal cortex layer-3 pyramidal neurons typically possess approximately 6,000 dendritic spines (Rocher et al., 2008). Dendritic spines are categorised based on their morphology into thin, stubby, mushroom, and cup-shaped (Harris et al., 1992). There is a however a dynamic change in spine morphology with presynaptic stimulation and in response to the surrounding environment. The morphology, structure and function of dendritic spines is modulated by a group of postsynaptic density (PDS) proteins which can be divided into four subgroups: scaffold proteins, receptor transmembrane proteins, cytoskeletal proteins and signalling proteins (Hering & Sheng, 2001; Verpelli et al., 2013). These proteins act in consort to ensure efficient signal transmission from the presynaptic membrane to the postsynaptic compartment, through the phenomenon of long-term potentiation - a functional correlate of dendritic spine plasticity which is dependent on the change in size and number of dendritic spines (Fu & Zuo, 2011).

The most abundant, and one of the widely studied, postsynaptic scaffolding proteins is PSD-95. It is localised very close to the postsynaptic membrane and thus can easily interact with receptors, ion channels and adhesion molecules, as well as with cytoplasmic proteins (Verpelli et al., 2013). It plays a key role in organising the signalling complexes at the postsynaptic membrane and is therefore critical to the function of the dendrite and long-term plasticity. Indeed, dysfunction of scaffolding proteins are key events in the pathophysiology of autism, schizophrenia and Alzheimer's disease. For example,

alterations in the expression of PSD-95 has been found in patients with Alzheimer's disease (Leuba et al., 2008) and in mouse models of Fragile-X syndrome with prominent cognitive and behavioural features (Zalfa et al., 2007). Similarly, deletion of the ProSAP2/Shank3 gene leading to absence of other scaffolding proteins, leads to Phelan-McDermid Syndrome presenting with significant dysfunction in multiple cognitive domains (Phelan & McDermid, 2012).

There are two key cytoskeletal components within the postsynaptic density, critical for dendritic structure and function: filamentous actin (F-actin), and microtubules (Fischer & Fowler, 2015). It is therefore not surprising that in neurodegenerative diseases with aggregation of the hyperphosphorylated microtubule-associated protein tau, microtubule dynamics is changed, causing changes in dendritic structure and function (Zempel & Mandelkow, 2014; Zempel et al., 2010). Moreover, mutations in the LRRK2 gene, that have recently been associated with predicting survival in PSP (Chen-Plotkin, 2021; Guerreiro et al., 2016), amongst many other pathological sequelae, are thought to be involved in microtubule fragmentation, and therefore dendritic dysfunction (Lin et al., 2010).

Loss of dendritic spine, and abnormal dendritic function in tauopathies, can therefore occur as a result of loss of afferent input secondary to presynaptic loss (as reviewed in Chapter 3), loss of the neuron- and axon-autonomous function, and also as a result of direct toxicity from hyperphosphorylated tau and tau oligomers in the extracellular space, and inflammation (Hoffmann et al., 2014; Kweon et al., 2017). Given that presynaptic density, and dendritic spine morphology are directly linked to synaptic function (Jähne et al., 2021; Yuste & Bonhoeffer, 2001), I propose that with the loss of synapses, presynaptic vesicle density as measured with [^{11}C]UCB-J, would correlate with changes in postsynaptic dendritic complexity, in PSP and CBD.

4.1.2 MEASURING DENDRITIC SPINES AND COMPLEXITY

The gold standard technique for studying dendritic spines *in vitro* is electron microscopy allowing the analysis of dendritic spine ultrastructure. Other techniques include immunohistochemistry using antibodies against various postsynaptic density proteins. The use of such techniques on, and interpretation of results from, donated human brain tissue is however limited given the rapid hypoxic-induced changes in dendritic spines soon after

death (Stewart et al., 2014). Furthermore *in vitro* methods do not allow correlations with disease severity in life, and the potential for therapeutic monitoring.

Microstructural integrity can be assessed *in vivo* using Diffusion Tensor Imaging (DTI). However, while diffusion tensor imaging (DTI) has proved to be a valuable non-invasive technique to probe microstructural white matter integrity, it has limited utility to characterise such changes in the cortex due to the isotropic water diffusion of the grey matter. To address the limitations of DTI, Neurite Orientation Dispersion Imaging (NODDI) has been developed. NODDI is a multi-compartment biophysical diffusion imaging model that is capable of disentangling the diffusion signal arising from distinct tissue compartments, such as extracellular, intracellular water and cerebrospinal fluid (H. Zhang et al., 2012). This multi-compartmentalisation of the diffusion signal enables metrics such as Neurite Density Index and Orientation Dispersion Index (ODI) to characterise the number of neurites as well as the variability of neurite orientations, respectively. Both the clinical feasibility and relevance of NODDI alterations have been successfully demonstrated in normal ageing and dementia (Cox et al., 2016; Parker et al., 2018; H. Zhang et al., 2012). However, the biological substrates of NODDI are still unclear due to limited evidence of cross-validation among NODDI metrics and alternative proxies of grey matter microstructure. Recent studies have shown that NODDI Orientation Dispersion Index (ODI) is correlated with histologically-derived indices of neurite dispersion (Grussu et al., 2017; Schilling et al., 2018), highlighting its potential viability as a proxy of underlying biological changes in grey matter microstructure. Of particular relevance to this chapter, reductions in cortical ODI has been found in a tau-transgenic mouse model, entirely consistent with the severe extent of dendritic degeneration (Colgan et al., 2016). There has not yet been an attempt to characterise the coupling of cortical ODI with measurements of synaptic pathology (with [^{11}C]UCB-J PET imaging). Compared to PET imaging, the ODI from NODDI would have the advantage of wide scalability in multicentre studies, repeatable assays without ionising radiation, and lower cost within a clinically feasible acquisition time (~ 15 minutes).

In this proof-of-concept study, I focus on progressive supranuclear palsy – Richardson's Syndrome (PSP) and Corticobasal Degeneration (CBD) as disease models. In PSP and CBD grey matter atrophy although present, is not extensive unlike in Alzheimer's disease where we would expect generalised atrophy; this allows us to examine cortical

microstructure without the influence of atrophy. I test the hypothesis that functionally relevant loss of presynaptic density caused by PSP and CBD is correlated to the changes in postsynaptic dendritic complexity, independent of changes in grey matter atrophy.

4.2 Methods and Study Design

The current experiment and the experiment reported in Chapter 3 were both part of the same protocol. Thus, the participants, inclusion and exclusion criteria, and study procedure overlap with those reported in Chapters 2 & 3. Compared to the cohort in Chapter 3, the current experiment featured twelve more control participants, five more patients with CBD, and eight more patients with PSP. Thus, the full sample available for the analysis of changes in Orientation Dispersion Imaging consisted of 27 controls, 14 patients with CBD, and 22 with PSP. Demographic details for this extended cohort are provided in Table 4-1.

4.2.1 Neuroimaging Pre-processing

4.2.1.1 T1-weighted MRI

T1-MPRAGE was acquired on high resolution Siemens Magnetom 3T PRISMA scanner (TE = 2.93 ms, TR = 2s, slice thickness = 1.1 mm , resolution = 1.1 mm^3 isotropic, 208 slices) (Table 2-2). The data were processed using Computational Anatomy Toolbox in SPM12. The T1-MPRAGE images were then segmented into grey matter, white matter, and CSF images by using a unified tissue segmentation technique after image intensity non-uniformity correction was performed. Regional cortical thickness was derived in CAT12 based on the projection-based thickness method (Dahnke et al., 2013) which uses topology correction (Yotter et al., 2011a) and spherical mapping (Yotter et al., 2011b). Previous studies have shown that CAT12 produces reliable estimates of cortical thickness, yielding larger effect sizes in case-control comparisons (Seiger et al., 2018). All segmentations were visually inspected by three colleagues. Three PSP-RS subjects were excluded due to sub-optimal contrast between grey and white matter tissue, resulting in unsatisfactory segmentations.

4.2.1.2 Diffusion-weighted MRI

Diffusion scans were acquired on Siemens Magnetom Prisma scanner (TE = 75.6ms, TR = 2.4s, slice thickness = 1.75mm, 98 directions, 104 slices, bvals = 300, 1000, 2000).

Diffusion datasets were pre-processed with FSL-FDT (FMRIB's Diffusion Toolbox). Firstly, the diffusion weighted-imaging data were stripped of nonbrain tissue using the Brain Extraction Tool. The resulting brain masks were visually inspected for anatomic fidelity. Eddy currents and head movements were corrected with "eddy" in FSL (Version 6.0.1). TOPUP was applied to correct for estimating and correcting susceptibility induced distortions. The b0 volume from the reversed phase-encode blip was used in TOPUP for the estimation and correction of susceptibility induced distortion. Quantitative identification of slices with signal loss was performed in "eddy" and these volumes were replaced by non-parametric predictions using the Gaussian process (J. L. R. Andersson et al., 2016). The b-matrix was subsequently reoriented by applying the rotational part of the affine transformation used during eddy correction (Leemans & Jones, 2009). Next, ODI maps were derived from the eddy-corrected datasets using the Microstructural Diffusion Toolbox (MDT) (Harms et al., 2017). There has been recent debate over the validity of NODDI's assumption of a fixed intrinsic diffusivity across the brain, especially in the grey matter. Therefore, to optimise analyses of ODI, the intrinsic diffusivity was set to 1.1×10^{-3} mm^2/s as previously proposed (Fukutomi et al., 2018).

4.2.1.3 [^{11}C]UCB-J Positron Emission Tomography (PET)

A subset of patients underwent dynamic PET imaging as well as NODDI (Controls = 19, PSP-RS = 20, CBD = 12 including those already included in Chapter 3) on a GE SIGNA PET/MR – the [^{11}C]UCB-J data acquisition is as described in 3.2.2.

4.2.1.4 Extraction of regional measurements from T1, ODI and [^{11}C]UCB-J BP_{ND}

ODI and [^{11}C]UCB-J maps were co-registered to the skull-stripped T1 brain image using rigid registrations in Advance Normalisation Tools (ANTS; http://stnava.github.io/ANTs/). The accuracy of all co-registrations was visually inspected. The co-registered ODI and [^{11}C]UCB-J volumes were projected to the surface for cortical analyses. To that end, a weighted-mean method was applied that uses a Gaussian kernel for mapping along the normal, based on the recommended settings in CAT12. Finally, region of interest (ROI) extraction was performed within the surface space using the Desikan Killiany atlas (Desikan et al., 2006). We used a modified Hammers atlas (Hammers et al., 2003) to extract measurements from the subcortical regions and midbrain. The atlas was subsequently transposed into the native spaces of ODI and [^{11}C]UCB-J respectively using the inverse of

transformations from the co-registrations between ODI or [^{11}C]UCB-J and T1 MPRAGE. Mean regional ODI, [^{11}C]UCB-J and grey matter volumes were extracted using *fslstats* in FSL. Finally, each subject has the following imaging measurements: regional cortical thickness, cortical ODI, cortical [^{11}C]UCB-J BP_{ND}, as well as subcortical and midbrain grey matter volumes, ODI and [^{11}C]UCB-J $BP_{ND.}$

4.2.2 Statistical Analysis

The software R was used to compare demographic variables between the diagnostic groups using ANOVA, Kruskal Wallis and chi-square tests where appropriate. To investigate regional group differences of grey matter, ODI and [^{11}C]UCB-J $BP_{ND,}$ a non-parametric permutation-based inference was used, implementing Permutation Analysis of Linear Models (PALM; https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/PALM) in MATLAB, adjusted for age (5000 permutations) (Winkler et al., 2014). A key advantage of permutation-based approach is the robustness of statistics to heteroscedasticity and its minimal assumptions on data distributions. Next, non-parametric permutation tests for Biological Parametric Mapping (BPM) (Casanova et al., 2007) were used to assess whole-brain inter-regional associations between ODI and [^{11}C]UCB-J BP_{ND} across the patient sample, as well as within CBD and PSP groups separately. Correlational analyses were adjusted for age and local grey matter thickness / volume. Statistical results were adjusted using the False Discovery Rate (FDR) correction across the cortical, subcortical regions and the midbrain (81 regions). Mean values, standard deviations, p values and Cohen's D were overlaid on 2D brain templates using the *ggseg* package in R (Mowinckel & Vidal-Piñeiro, 2020).

4.3 RESULTS

4.3.1 DEMOGRAPHICS

Clinical and demographic information are summarised in Table 4-1. Patients and controls were matched in age and gender although in this larger group of patients compared to those in chapter 3, education years was significantly lower in PSP relative to controls (Post-hoc Dunn's Test after Kruskal-Wallis test; $p < 0.001$). Patients with PSP and CBD had lower total ACE-R scores compared to controls as in the smaller cohort in Chapter 3 ($p < 0.001$).

	Control	CBD	PSP	P values
M:F	16:11	9:5	10:12	ns[a]
Age	69.0 (7.34)	70.0 (7.91)	70.9 (8.69)	ns
Education in years	14.9 (3.65)	12.4 (3.03)	12.1 (4.33)	0.005
ACE-R total (max. 100)	95.9 (3.81)	78.2 (18.9)	80.4 (12.7)	<0.001
PSPRS (max. 100)	0.148 (0.534)	26.9 (7.88)	33.3 (9.86)	<0.001
NODDI MRI - [^{11}C]UCB-J PET scan interval (days)	255.5 (168.9)	119.1 (109.8)	167.0 (134.7)	0.04

Table 4-1. Demographics and neuropsychological profile for each participant cohort.
Abbreviations: CBD = Corticobasal Degeneration defined as amyloid negative corticobasal syndrome; PSP-RS = Progressive Supranuclear Palsy - Richardson's syndrome; PSPRS = Progressive Supranuclear Palsy Rating Scale; ACER = Addenbrooke's Cognitive Examination-Revised. Parameters are expressed as mean ± standard deviation. P values are shown for analysis of variance across groups or chi-squared[a] test as appropriate.

4.3.2 GROUP COMPARISONS OF GREY MATTER ATROPHY, ODI AND [^{11}C]UCB-J BP_{ND}

For all groups, mean ± standard deviation (SD) distributions of regional cortical thickness, subcortical grey matter volumes, ODI and [^{11}C]UCB-J BP_{ND} are overlaid on brain templates in

Figure 4-1 (cortical) and Figure 4-2 (subcortical)**.** Permutation-based statistical comparisons across all imaging measurements are reported for PSP and CBD relative to controls, adjusted for age and FDR corrected, in Figure 4-3 and Figure 4-4.

4.3.2.1 Progressive supranuclear palsy

Statistical results and corresponding effect sizes from the comparisons between PSP and controls are illustrated in Figure 4-3A-C. Non-parametric permutation-based tests showed that, relative to controls, the PSP group exhibited significant cortical thinning particularly in the motor cortex and frontal cortices; subcortical atrophy was found in the thalamus,

putamen and pallidum and midbrain. In PSP, significant reductions in cortical ODI were more widespread than areas affected by atrophy, including multiple areas within the cortical mantle and also subcortically including the basal ganglia. Post-hoc paired t-tests of the regional Fisher's transformed Cohen's D indicated that the regional effect sizes were significantly larger for ODI compared to grey matter atrophy (Figure 4-3C)**.** As expected, the PSP group showed extensive and severe reductions of [^{11}C]UCB-J BP_{ND} across the cortex and subcortical regions compared to controls, replicating findings in Chapter 3, here in a larger sample size. Across the whole brain, reductions in [^{11}C]UCB-J BP_{ND} yielded the largest effect size relative to both ODI and grey matter atrophy.

4.3.2.2 Corticobasal Degeneration

Statistical results and corresponding effect sizes from the comparisons between CBD and controls are illustrated in Figure 4-4A-C**.** Relative to controls, the CBD group showed focal cortical thinning in motor cortex, superior frontal cortex and the occipital lobe, as well as atrophy in the left putamen and bilateral pallidum. In contrast, widespread ODI reductions were found extending beyond the atrophy-affected motor cortices to other regions that were relatively preserved from atrophy, notably the temporo-parietal and cingulate cortices. In addition, there was bilateral significant ODI reductions in the caudate and putamen, both of which showed no significant grey matter atrophy. Accordingly, regional Cohen's D of ODI was significantly larger than that of grey matter atrophy (Figure 4-4C). Finally, [^{11}C]UCB-J BP_{ND} comparisons revealed a generalised and widespread extent of significantly reduced [^{11}C]UCB-J BP_{ND} across the cortex and subcortical regions compared to controls, replicating results in Chapter 3; similar to the PSP cohort, reductions in [^{11}C]UCB-J BP_{ND} yielded the largest effect size relative to both ODI and grey matter atrophy (Figure 4-4C).

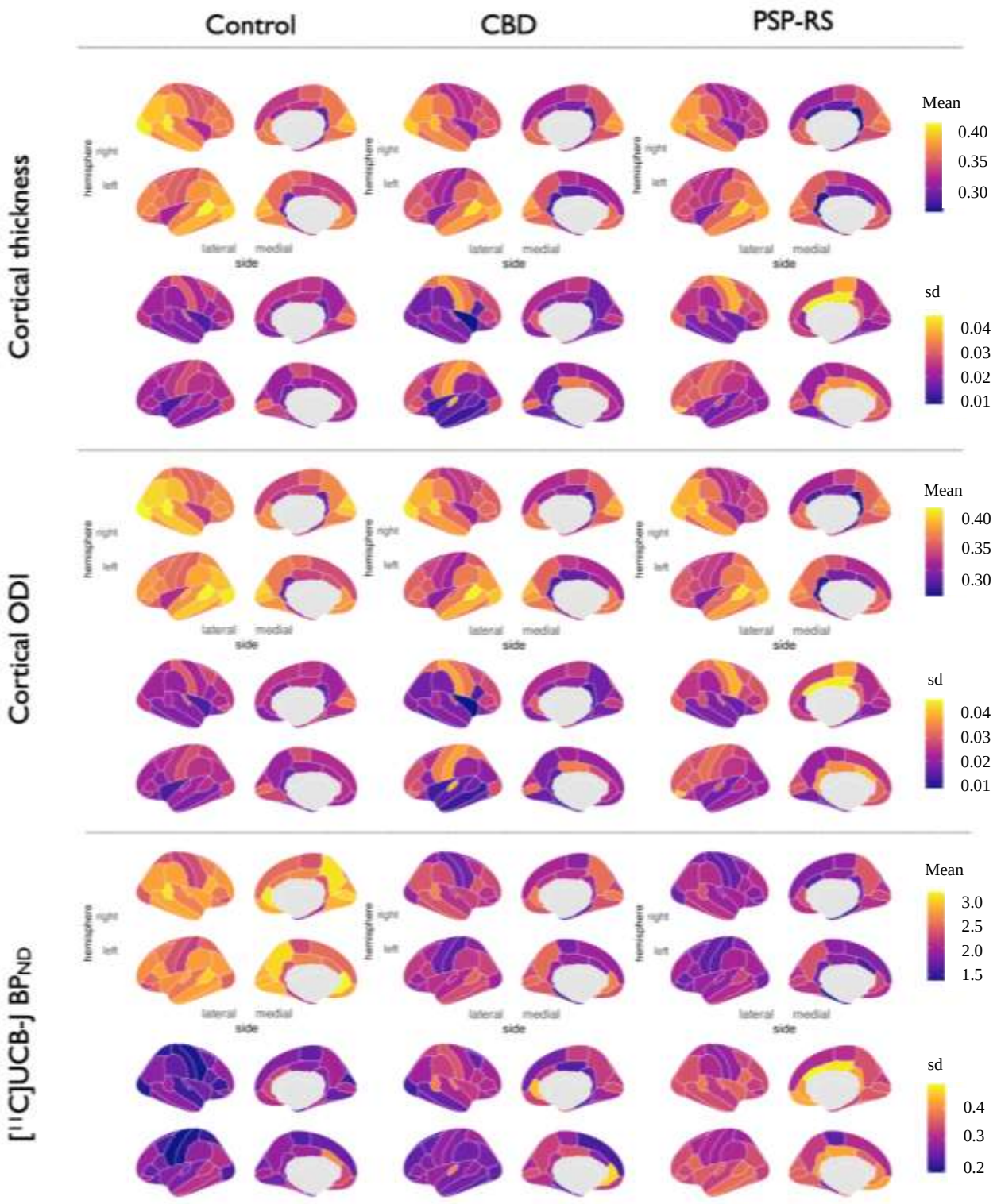

Figure 4-1. Regional distributions of mean (sd) cortical thickness, cortical ODI and cortical [^{11}C]-UCB-J BP_{ND} across controls, CBD, and PSP.
Abbreviations: ODI = Orientation Dispersion Index, CBD = amyloid negative Corticobasal Syndrome; PSP = Progressive Supranuclear Palsy-Richardson's Syndrome; sd = Standard deviation.

Figure 4-2. Subcortical (basal ganglia and brainstem) distributions of mean (sd) grey matter volumes, ODI and [^{11}C]-UCB-J BP_{ND} across controls, CBD, and PSP.
Abbreviations: ODI = Orientation Dispersion Index, CBD = amyloid negative Corticobasal Syndrome; PSP = Progressive Supranuclear Palsy-Richardson's Syndrome; sd = standard deviation; GM = grey matter.

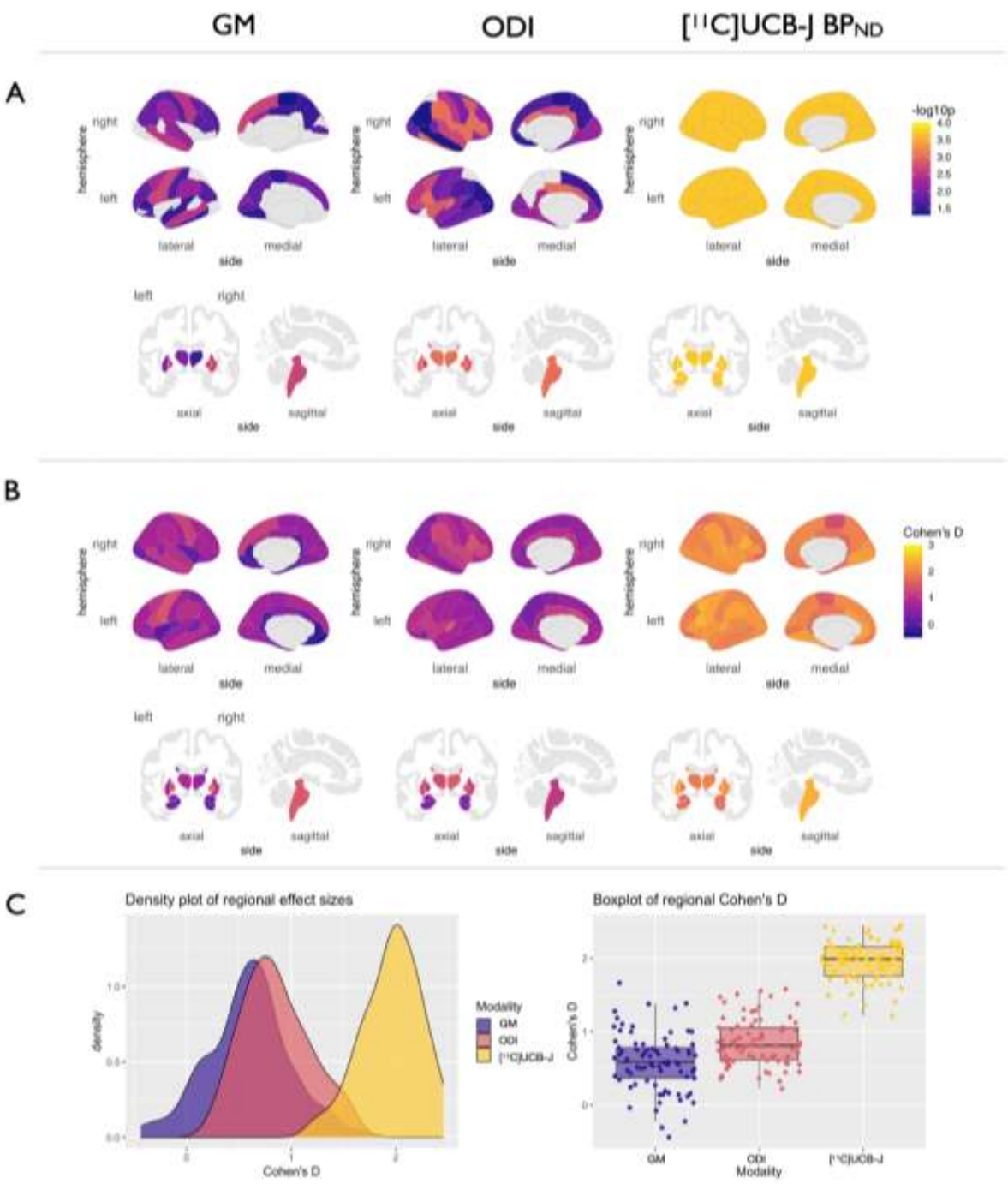

Figure 4-3. GM atrophy, reduced ODI and [^{11}C]UCB-J BP_{ND} in PSP vs Controls.
A: p-values of regions surviving FDR correction are visualised on cortical and subcortical brain templates showing reduced ODI and [^{11}C]UCB-J binding beyond atrophic areas. B: The regional effect sizes (Cohen's D) for the regional reductions in GM, ODI, [^{11}C]UCB-J BP_{ND} compared to controls. C: Density plots of distributions of effect sizes and boxplots of effect sizes of grey matter atrophy, ODI and [^{11}C]UCB-J BP_{ND} from the group comparisons showing significantly larger effect sizes for presynaptic loss compared to ODI and atrophy. Abbreviations: ODI = Orientation Dispersion Index; PSP = Progressive Supranuclear Palsy-Richardson's Syndrome; FDR = False Discovery Rate; GM = grey matter.

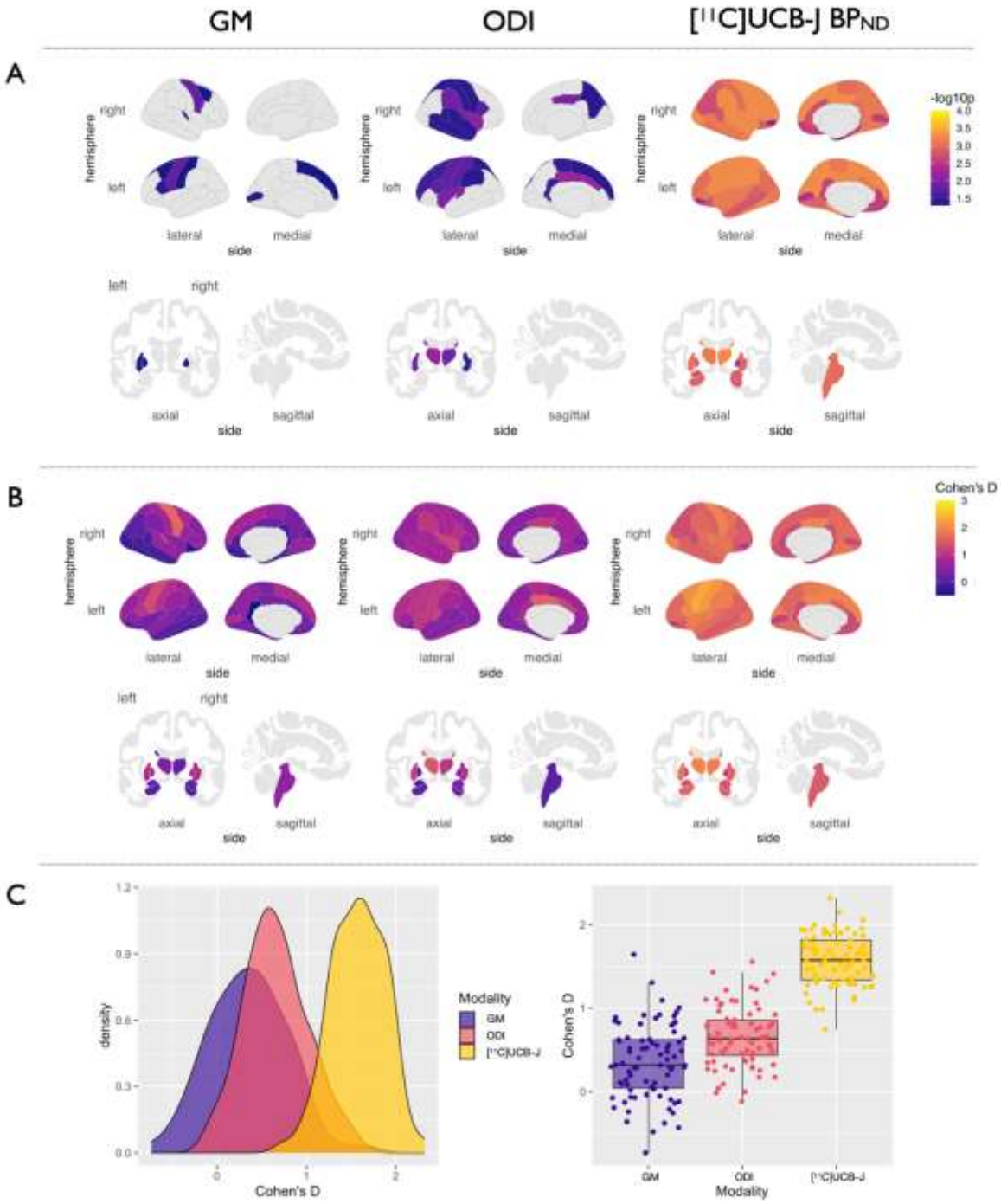

Figure 4-4. GM atrophy, reduced ODI and [^{11}C]UCB-J BP_{ND} in CBD vs Controls.
A: P-values of regions surviving FDR correction are visualised on cortical and subcortical brain templates B: The effect sizes (Cohen's D) for the regional reductions in GM, ODI, [^{11}C]UCB-J BP_{ND} compared to controls. C: Density plots of distributions of effect sizes and boxplots of effect sizes of grey matter atrophy, ODI and [^{11}C]UCB-J BP_{ND} from the group comparisons showing significantly larger effect sizes for presynaptic loss compared to ODI and atrophy. Abbreviations: ODI = Orientation Dispersion Index; FDR = False Discovery Rate; GM = grey matter; CBD = amyloid negative Corticobasal Syndrome.

4.3.3 REGIONAL ASSOCIATION OF ODI WITH [^{11}C]UCB-J BP_{ND}

Non-parametric permutation models were used to assess the inter-regional associations between ODI and [^{11}C]UCB-J BP_{ND} across the full patient sample, while sensitivity analyses were conducted for PSP and CBD groups separately. To visualise the spatial patterns of these regional correlations, accounting for age, regional grey matter atrophy and NODDI/UCB-J PET scan interval, the p values are projected on brain templates (

Figure 4-5**).** Within the total sample of CBD and PSP patients, multiple cortical and subcortical regions demonstrated local positive associations where [^{11}C]UCB-J BP_{ND} locally predicted ODI, i.e. across subjects, both presynaptic density and dendritic complexity showed reciprocal associations within the same region (

Figure 4-5A). Scatter plots for each significant local association are shown in supplementary Figure B1. The regions showing the strongest correlations between ODI and [^{11}C]UCB-J BP_{ND} were primarily in bilateral pre- and postcentral gyri and the prefrontal cortex, but also extended to subcortical areas including the basal ganglia, thalamus and the midbrain. When the analyses were restricted to the CBD sample, the spatial extent of local associations was markedly attenuated, although significant associations were still present within the bilateral pre- and postcentral gyri, and isolated regions in the frontal and occipital lobe

Figure 4-5B**.** Within the PSP group, [^{11}C]UCB-J BP_{ND} was significantly correlated with ODI in a widespread spatial pattern similar to that of the total sample. Peak correlations were identified within the motor cortex and cingulate regions (

Figure 4-5C)**,** but also included the thalami, midbrain and parts of the basal ganglia**.** These analyses did not include the control group and are thus not indicative of a group effect. Nevertheless, to determine the specificity of the coupling between ODI and [^{11}C]UCB-J BP_{ND}, the same non-parametric permutation model was performed on the controls. This analysis did not yield any significant local associations that retained statistical significance after FDR correction.

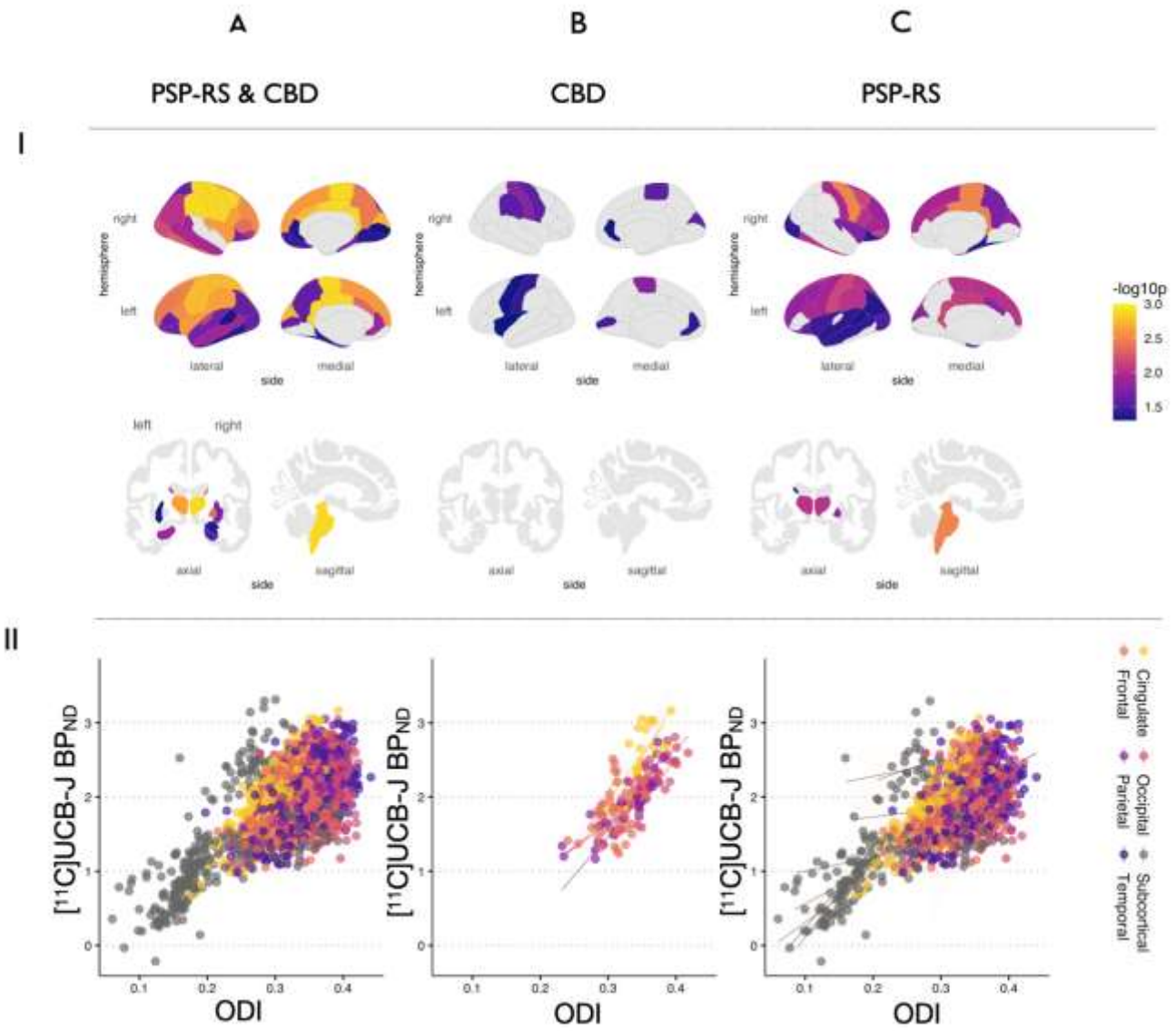

Figure 4-5. Local associations between ODI and [^{11}C]UCB-J BP_{ND}.
(A) total sample of patients, (B) CBD and (C) PSP, separately. I: Statistical p values for the regional association of [^{11}C]UCB-J BP_{ND} and ODI, for regions surviving FDR correction, are overlaid on cortical and subcortical brain templates. II: Scatter plots showing the relationships between ODI and [^{11}C]UCB-J BP_{ND} for all regions surviving FDR correction, coloured by lobes and subcortex. Abbreviations: ODI = Orientation Dispersion Index. PSP = Progressive Supranuclear Palsy-Richardson's Syndrome; CBD = amyloid negative Corticobasal Syndrome.

4.4 DISCUSSION

In this chapter I tested the hypothesis that changes in presynaptic density are closely linked to MRI measures of cortical microstructure, in the neurodegenerative tauopathies of CBD and PSP. The main insights are that (i) in the neurodegenerative tauopathies of CBD and PSP, widespread changes in grey matter dendritic complexity are observed even in areas without significant atrophy; and (ii) NODDI ODI, an MRI-based measure of postsynaptic dendritic structure and complexity, correlates with the loss of presynaptic density estimated by the PET radioligand [^{11}C]UCB-J; this effect is not a result of changes in grey matter atrophy. The findings extend previous investigations of ODI microstructural changes in PSP and CBS/CBD (Mitchell et al., 2019), demonstrating the *in vivo* coupling of dendritic complexity to presynaptic density, in line with preclinical models of tauopathies (Hoffmann et al., 2014; Rocher et al., 2010).

Grey matter atrophy within the motor cortex, as well as the basal ganglia and brainstem are common MRI findings in PSP and CBD; the pattern of atrophy shown in Figure 4-1 and Figure 4-2 concords with previous studies (Jabbari et al., 2019). In this chapter however, I show that both within areas of the brain where there is atrophy, and in areas with absent atrophy, there is significant and more severe loss of dendritic complexity and, as previously shown in Chapter 3 and replicated here, presynaptic density in the patient cohort. The widespread pattern of cortical ODI and presynaptic deficits in PSP and CBD matches the expected pattern of tau pathology, both in the subcortical and cortical areas (Dickson et al., 2011; Kovacs et al., 2020), and concords with preclinical models of tauopathy. For example, in a P301S mouse model of tauopathy, using confocal microscopy of hippocampal and medial frontal cortical dendrites, Walker *et al.* have shown age-related vulnerability to dendritic spine remodelling and reductions in the hippocampus and medial prefrontal cortex, respectively, in response to tau pathology (C. K. Walker et al., 2021).

Loss of dendritic complexity and presynaptic loss, in non-atrophied areas of the brain, for example the occipital lobes, potentially reflects early changes in synaptic function, in response to tau pathology or toxic oligomers of tau, that later in the disease process progress to atrophy. Indeed, in preclinical models, pathological tau oligomers (Kaniyappan et al., 2017; Polydoro et al., 2014; Usenovic et al., 2015; Yasumasa Yoshiyama et al., 2007)

induce synaptic degeneration rather than neurofibrillary tangles (Kuchibhotla et al., 2014), and interfere with synaptic function and density, in the absence of neuronal loss.

The effect size for the comparison between ODI and [^{11}C]UCB-J BP_{ND} in patients relative to controls, was stronger than for atrophy. Together, the larger extent of ODI reductions relative to grey matter atrophy indicate that NODDI can reveal new aspects of cellular pathology in tauopathies, in keeping with the recent demonstration that changes in ODI parameters closely reflect complex histological changes (Grussu et al., 2017). Notwithstanding the cross-sectional design of this experiment, this observation highlights the early structural changes in disease pathogenesis that may underlie the emergence of cognitive and motor symptoms not attributable to atrophy. Indeed, human studies of cortical physiology in PSP, and animal models of tauopathy have illustrated abnormal electrophysiology in the absence of atrophy (Hughes et al., 2013; Sami et al., 2018) or neuronal loss (Menkes-Caspi et al., 2015). The findings of reduced ODI beyond atrophy are in agreement with the literature in the related tauopathy of Alzheimer's disease (Parker et al., 2018) where there is a stronger relationship between cognitive function and synaptic density than with atrophy (Masliah et al., 1992; Scheff et al., 2006; Terry et al., 1991).

The observation of a tight coupling between dendritic complexity, as measured with ODI, and presynaptic density, as measured with [^{11}C]UCB-J, in the patient cohort, echoes preclinical findings in animal models of tauopathy (Dorostkar et al., 2015; Harris & Kater, 1994; Herms & Dorostkar, 2016; Kweon et al., 2017), and post mortem studies (Bigio et al., 2001; Lipton et al., 2001). There are two potential explanations for this tight coupling: first, tau protein is enriched in axons and associated with axonal growth and transport; the synaptic toxicity associated with pathological tau is observed both in pre- and postsynaptic structures (Kweon et al., 2017; Y. Wang & Mandelkow, 2012), leading to both a reduction in dendritic complexity and presynaptic density. Second, while a cross-sectional design precludes inferences of causality, alterations in presynaptic function in CBD and PSP are expected to induce dendritic morphological alterations with early loss of dendritic spines and reduced dendritic branching. This has been illustrated in animal studies, where postsynaptic dendritic morphology correlates with the numbers of presynaptic vesicles and synaptic strength (Schikorski & Stevens, 1999). It is possible that primary dendritic degeneration in CBD and PSP causes a reduction in the density of the presynaptic contacts on the distal dendrites. These alternative accounts are not mutually exclusive and could act

in parallel to impair effective transneuronal connectivity, and subsequently motor and cognitive function.

The downstream effect of the above is a loss of functional connectivity. Indeed in PSP there is reduced resting state connectivity between cortical and subcortical areas (reviewed in Filippi et al. (2019)). In CBD, the evidence is more heterogenous given the mixed underlying pathology, and the lack of categorising amyloid status in previous functional studies (also reviewed in Filippi et al. (2019)).

Synaptic pathology is a common feature in many neurodegenerative diseases (Clare et al., 2010; Herms & Dorostkar, 2016; Kweon et al., 2017). In the related tauopathy of Alzheimer's disease, widespread reduction in synapses have been shown using [^{11}C]UCB-J PET in patients (M. K. Chen et al., 2018; Mecca et al., 2020), as well as changes to dendritic complexity seen both *in vivo* as reduced ODI (Parker et al., 2018) and in animal models of Alzheimer's disease (Dorostkar et al., 2015). Similar pre- and postsynaptic losses are seen in the substantia nigra of patients with Parkinson's disease (Bellucci et al., 2016; Reeve et al., 2018), and within the hippocampi of patients with Lewy Body Dementia (Revuelta et al., 2008). The tight coupling of the presynaptic and postsynaptic compartments shown in here, may therefore extend to pathologies other than primary 4-repeat tauopathies, where synaptic dysfunction is one of the earliest stages of disease, regardless of the culprit protein aggregate in question. If so, the opportunity to utilise both MRI and PET to understand disease pathogenesis would greatly facilitate cohort studies of individual phenotypic differences or repeat testing in clinical trials monitoring. Furthermore, the *in vivo* observation of the tight coupling between the pre- and postsynaptic compartments, offers a new angle of interpretation for future studies utilising either of these methods in isolation. For example if cost, scalability and resources are limited for larger cohort studies, MRI measures of dendritic complexity can offer a close surrogate of presynaptic density, although I appreciate it is not a substitute given the significantly larger effect sizes seen with [^{11}C]UCB-J (Figure 4-3C and Figure 4-4C) . This latter observation may indicate a more severe toxic effect at the pre-synapse compared to dendritic pathology, although longitudinal studies are required to confirm this. However, it is important to note that ODI should not be taken as a direct representation of the postsynaptic density seen in histological studies. Rather, it provides complementary information to the PET data, with

support from histological studies that have shown ODI to be a close surrogate (Grussu et al., 2017).

There are several strengths to the experimental procedures in this chapter. First, the NODDI resolution of 1.75mm was specifically optimised for the investigation of cortical microstructure, and is higher than previous NODDI studies in early onset AD (2.5 mm) (Parker et al., 2018) , Parkinson's disease (5 mm) (Kamagata et al., 2016) and PSP (2.0 mm) (Mitchell et al., 2019) . Second, I use amyloid imaging to exclude patients with CBS likely due to Alzheimer's disease. This helps in reducing the potential underlying pathologies at play in the CBS cohort to predominantly 4-repeat tauopathies, although I acknowledge other pathologies are possible but less likely (Alexander et al., 2014) – in this regard it is reassuring that the spatial patterns of reductions in ODI and [^{11}C]UCB-J binding are not substantially different between the PSP and CBD cohorts. Thirdly, the use of non-parametric permutation analyses also confers additional robustness to the statistical results, since this approach is not reliant on data distributions (Winkler et al., 2014).

I acknowledge the limitations of this experimental chapter including the relatively small sample size in the CBD cohort. However, given the large effect sizes seen of ODI and [^{11}C]UCB-J binding reduction in both patient groups, the study was sufficiently powered to examine the relationships between both imaging markers. Secondly, I acknowledge that the MRI measure of ODI is not a direct measure of postsynaptic density but rather dendritic complexity; PET radioligands targeting the postsynaptic density may therefore provide further useful insights into postsynaptic pathology. Furthermore, although a tight correlation between our two measures of synaptic health is illustrated, I do not show causality in this cross-sectional study design.

Understanding the pathological processes that precede atrophy in neurodegeneration is key not only in expanding our knowledge of the pathophysiology of disease but also in informing the design of clinical trials both in terms of the imaging options for measuring disease-related changes, and the optimal timing of intervention in the disease process. The data presented in this chapter implicate correlated changes in dendritic microstructure and synaptic density in patients with primary degenerative tauopathies including PSP and CBD. Further cross-validation of ODI with [^{11}C]UCB-J BP_{ND} may help further our understanding

of the pathophysiology of neurodegeneration, applicable to future studies of early neurodegeneration with a safe and widely available MRI platform.

5 THE RELATIONSHIP BETWEEN *IN VIVO* MOLECULAR PATHOLOGY AND SYNAPTIC LOSS IN PRIMARY TAUOPATHIES: A COMBINED [^{18}F]AV-1451 AND [^{11}C]UCB-J PET STUDY.

A manuscript related to this chapter is currently under revision as:

Holland N, Malpetti M, Rittman T, Mak E, et al. The relationship between molecular pahtology and synaptic loss in the primary tauopathies: a combined [^{18}F]AV-1451 and [^{11}C]UCB-J PET study.

The original data, on which the analyses in this chapter are based, were collected by myself, Dr George Savulich and Mrs Julie Wiggins. Patients were scanned and assessed as part of the Synaptic and Tau Evaluation in NeuroDegeneration Research (**S**ENDeR and **T**ENDeR) studies which were conceived by Professor James Rowe and Professor John O'Brien. The PET data were pre-processed by Dr Tim Fryer and Dr Young Hong. I designed the analysis strategy, performed the analyses, constructed the figures, and wrote the manuscript.

5 | The relationship between in vivo molecular pathology and synaptic loss in primary tauopathies: a combined [^{18}F]AV-1451 and [^{11}C]UCB-J PET study.

Summary

The relationship between *in vivo* synaptic density and tau burden in primary tauopathies is key to understanding the impact of tauopathy on functional decline and in informing new early therapeutic strategies. In this chapter, I determine the *in vivo* relationship between synaptic density and molecular pathology, in the primary tauopathies of Progressive Supranuclear Palsy (PSP) and Corticobasal Degeneration (CBD), as a function of disease severity.

Twenty-three patients with PSP, and twelve patients with amyloid-negative Corticobasal Syndrome (CBS) were recruited from a tertiary referral centre. Nineteen education, sex- and gender-matched control participants were recruited from the National Institute for Health Research 'Join Dementia Research' platform. Cerebral synaptic density and molecular pathology, in all participants, were estimated using PET imaging with the radioligands [^{11}C]UCB-J and [^{18}F]AV-1451, respectively. Patients with CBS also underwent amyloid PET imaging with [^{11}C]PiB to exclude those with likely Alzheimer's pathology – I refer to the amyloid negative cohort as having CBD. Disease severity was assessed with the PSP rating scale; regional non-displaceable binding potentials (BP_{ND}**)** of [^{11}C]UCB-J and [^{18}F]AV-1451 were estimated in regions of interest from the Hammersmith Atlas, excluding those with known off-target binding for [^{18}F]AV-1451. As an exploratory analysis, I also investigated the relationship between molecular pathology in cortical brain regions, and synaptic density in subcortical areas. The patient data were normalised against the controls, in all regions of interests.

Across brain regions, there was a *positive* correlation between [^{11}C]UCB-J and [^{18}F]AV-1451 BP_{ND} (β=0.3, t=4.7, p<0.0001)**.** However, the direction of this correlation became less positive as a function of disease severity in patients (β = -0.01, T = -2.2, p = 0.03). Between brain regions, cortical [^{18}F]AV-1451 binding was *negatively* correlated with synaptic density in subcortical areas (nucleus accumbens, caudate nucleus, and putamen).

Brain regions with higher synaptic density are associated with a higher [^{18}F]AV-1451 binding in PSP/CBD, but this association diminishes with disease severity. Moreover, higher cortical [^{18}F]AV-1451 binding correlates with lower subcortical synaptic density. Longitudinal imaging is required to confirm the mediation of synaptic loss by molecular

pathology. However, the effect of disease severity suggests a biphasic relationship between synaptic density and tauopathy, with synapse rich regions vulnerable to accrual of pathology, followed by a loss of synapses in response to pathology. Given the importance of synaptic function for cognition, the findings here elucidate the pathophysiology of primary tauopathies and may inform the design of future clinical trials.

5 | The relationship between in vivo molecular pathology and synaptic loss in primary tauopathies: a combined [^{18}F]AV-1451 and [^{11}C]UCB-J PET study.

5.1 INTRODUCTION

Synaptic loss is a feature of many neurodegenerative disorders. In chapters 3 and 4, I reviewed the critical role of synaptic dysfunction in the pathogenesis and cognitive impairment in PSP and CBD, which can begin long before, and is more extensive than neuronal loss (DeKosky & Scheff, 1990; Jacobsen et al., 2006; Terry et al., 1991). However, the *in vivo* relationship between synaptic loss and the underlying proteinopathy in primary tauopathies, tau, remains to be determined.

5.2 TAU-INDUCED TOXICITY IN TAUOPATHIES

The neurofibrillary tangles associated with PSP are postulated to begin within the pallidonigrolusyian system, spreading to the basal ganglia, pontine nuclei, and cerebellar dentate nucleus early in the disease process, before spreading into other cortical areas (Figure 5-1) (Kovacs et al., 2020). However as shown in chapter 3, significant synaptic loss is seen even in areas of the brain that are affected later in the disease stage, for example the occipital lobes. Whilst loss of cortico-cortical connections can account for this, an alternative explanation is that tau oligomeric spread to these areas precede tau tangles and lead to synaptic toxicity (DeVos et al., 2018). This is in accordance with preclinical models where synaptotoxicity of oligomeric tau leads to reduced synaptic plasticity and density (DeVos et al., 2018; Niewiadomska et al., 2021; Pickett et al., 2017; Ward et al., 2012). In tauopathies, mutant tau is present not only in the axons, but also the cell body and dendrites. As such, the mechanisms by which synaptic loss occurs following tau pathology are both direct and indirect (reviewed in Jadhav et al. (2015) and (Spires-Jones & Hyman, 2014)), but can also occur in response to the underlying genetic risk factors. For example, in patients with mutations of microtubule-associated protein tau (MAPT), there are deficiencies in many synaptic pathways including GABA-mediated signalling and synaptic plasticity (Jiang et al., 2018).

5 | The relationship between in vivo molecular pathology and synaptic loss in primary tauopathies: a combined [18F]AV-1451 and [11C]UCB-J PET study.

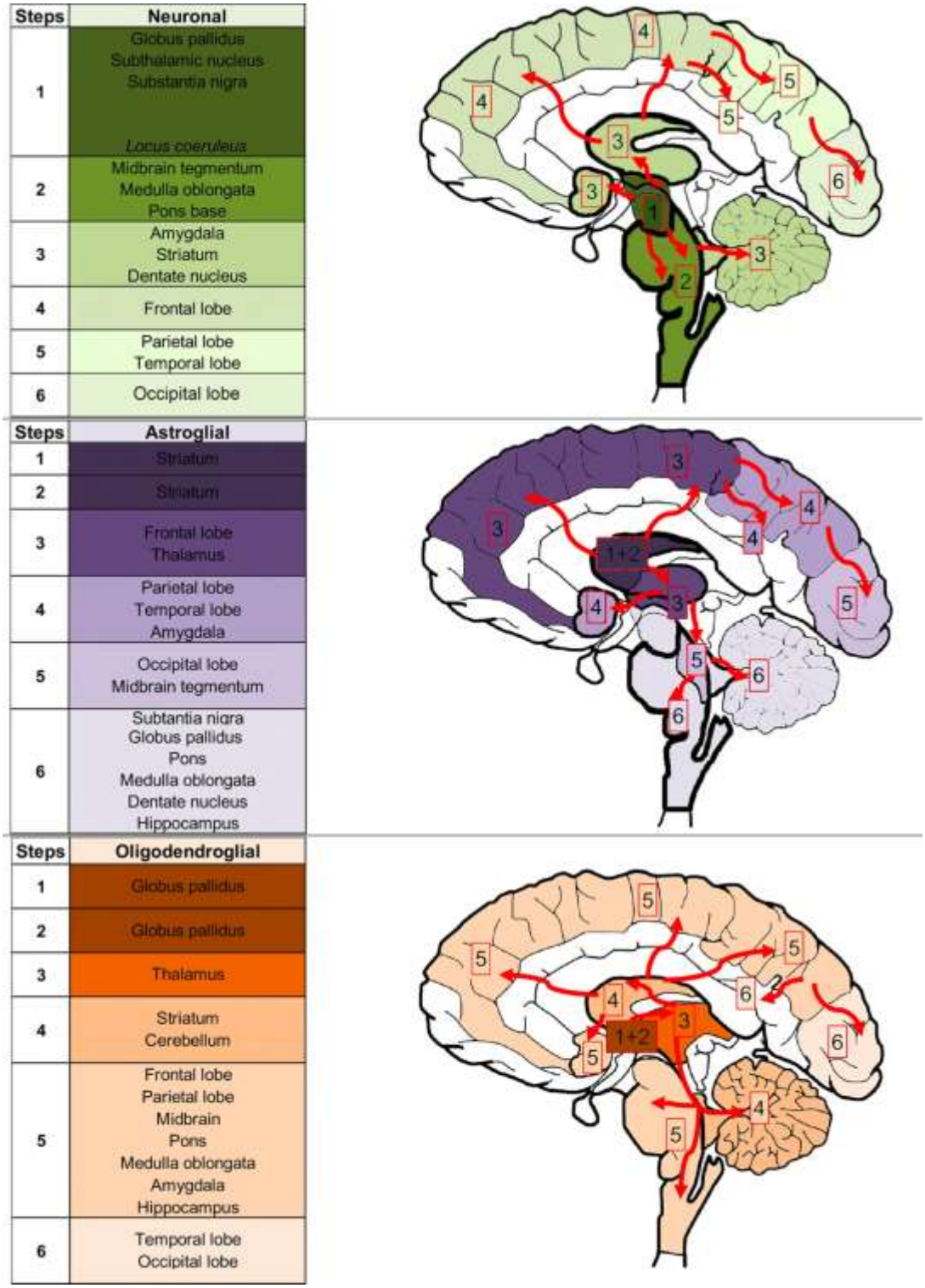

Figure 5-1. The neuropathological distribution of neurofibrillary tangles in different stages of PSP. Sequences of PSP related tau pathology based on the distribution of neuronal, astroglial, and oligodendroglial tau pathology. In Stage one disease, although the predominant findings are that of subcortical neuronal tau accumulation, pathological astroglial tau is present within the frontal lobes and can therefore alter synaptic physiology indirectly. Figure reproduced from Kovacs *et al.* 2020. with permission from Springer.

5.2.1 DIRECT TAU SYNAPTO-TOXICITY

Under normal physiological conditions, neuronal tau is involved in maintaining synaptic integrity and maintaining dendritic spine and axonal structure (as reviewed in 1.1.5.1). Under pathological conditions, it directly interferes with the function of many synaptic vesicle proteins including synaptophysin, synaptotagmin and synaptogyrin-3, leading to reduced synaptic vesicle mobility and therefore neurotransmitter release (Hanger et al., 2019). At the postsynaptic membrane, overexpression of tau in the P301L transgenic mouse model of tauopathy significantly interferes with the functioning of the postsynaptic density proteins, and thus the structure and integrity of dendritic spines (Regan & Cho, 2019).

5.2.2 INDIRECT TAU SYNAPTO-TOXICITY

Given the key role of tau as a microtubule-associated protein, under pathological conditions, hyperphosphorylated tau interferes with axonal cytoskeletal structure and thus the transport of key cargoes (for example synaptic vesicle proteins, mitochondria, and ion channels) to the cell body and axon terminal. This disruption to the normal transport machinery leads to a lack of vital molecules for the normal functioning of synapses (Kneynsberg et al., 2017). Pathological tau also interferes with normal functioning of mitochondria (particularly of complex I), in doing so it has a deleterious effect on calcium homeostasis (and therefore neurotransmitter release), and postsynaptic dendritic integrity (Eckert et al., 2014; Kopeikina et al., 2011; Lasagna-Reeves et al., 2011a; Yu et al., 2017).

Despite the physiological function of tau as an intracellular microtubule-associated protein, various species of tau have been observed in the extracellular environment, the so-called extracellular tau, albeit in smaller concentrations. Under normal physiological conditions, extracellular tau is found in the interstitial fluid and the cerebrospinal fluid (CSF) of mice (Yamada et al., 2011), and in human brains (Magnoni et al., 2012). Extracellular tau is also found in the hyperphosphorylated form in the CSF of patients with PSP and CBD, where reductions in the CSF are directly correlated with the severity and rate of disease progression in PSP (Rojas et al., 2018). In its hyperphosphorylated form, extracellular tau can cause synaptic toxicity in three ways: first, by directly interfering with calcium homeostasis, and therefore synaptic function (Gómez-Ramos et al., 2006; Gómez-Ramos et al., 2008), leading to impaired memory (Lasagna-Reeves et al., 2011b). Indeed clearance

of extracellular tau oligomers can reverse the deleterious effect on memory and locomotor deficits in mouse models of 4-repeat tauopathy with the P301L-MAPT mutation (Castillo-Carranza et al., 2014). Secondly, extracellular tau pathology can indirectly affect synaptic function through inflammation-induced synaptic toxicity (Reid et al., 2020). Mouse models of tauopathies support the idea that both extracellular tau pathology as well as astrocytic tau pathology contribute to glial degeneration, as a consequence leading to synaptic degeneration and loss (Amro et al., 2021; Blutstein & Haydon, 2014; Haydon, 2001; Kovacs, 2020; Vogels et al., 2019). Thirdly, ongoing synaptic toxicity can result from the intercellular propagation of pathology, which I review next.

5.3 TRANS-SYNAPTIC SPREAD OF TAU

Increasing evidence suggests that tau pathology can be transmitted from one cell to another through intercellular transmission in both *in vitro* and *in vivo* animal models (Clavaguera et al., 2009), following a prion-like hypothesis of spread (Goedert et al., 2017b; B. B. Holmes & Diamond, 2014) . This process is dependent on intracellular tau released from the presynaptic membrane, the uptake of extracellular tau, and ongoing aggregate formation within the recipient cell through recruitment of endogenous tau ('seeding'). It has been suggested that under normal physiological conditions, tau is actively secreted predominantly by ectosomes, and a shift towards exosomal secretion occurs upon increasing cellular tau accumulation (Dujardin et al., 2014). However, hyperphosphorylated tau can also directly translocate through the plasma membrane into the extracellular space (Katsinelos et al., 2018). This process is accelerated in response to lysosomal dysfunction, nutrient deprivations, and changes in Golgi dynamics and membrane trafficking properties (Mohamed et al., 2017; Mohamed et al., 2014). Once in the extracellular space, tau is taken up by recipient cells either via bulk endocytosis (Evans et al., 2018; B. B. Holmes et al., 2013), receptor-mediated endocytosis (Gómez-Ramos et al., 2009; Lasagna-Reeves et al., 2012), through tunnelling nanotubes (Tardivel et al., 2016) or extracellular vesicle shuttles (Guix et al., 2018). Once in the recipient cell, pathologic tau has the ability to induce seeding (Guo & Lee, 2011; Mirbaha et al., 2015).

Although tau is toxic to synapses, the spread of tau pathology is dependent on synaptic connections. This concept has been confirmed both in animal models of Alzheimer's

disease (DeVos et al., 2018; Seemiller et al., 2021) and in the P301L mouse model of primary tauopathy (Ahmed et al., 2014; Polanco et al., 2018). In this respect seemingly distant neurons can affect each other's synaptic transmission if they are synaptically connected (for example cortical-subcortical connections, or cortico-cortical connections).

In clinical disorders, tau burden can be characterised by PET imaging, using a variety of radiotracers (Y. T. Wang & Edison, 2019). In patients with amyloid positive mild cognitive impairment for example, increased temporal lobe binding of the tau radioligand [^{18}F]MK-6240 is associated with decreased synaptic density measured by the radioligand [^{11}C]UCB-J (Vanhaute et al., 2020). Similarly, in patients with Alzheimer's disease, Coomans et al, report reduced [^{11}C]UCB-J uptake, with increased uptake in the tau tracer [^{18}F]AV-1451, but within subjects this relationship is modulated by the individual's neocortical tau load (Coomans et al., 2021). However, the pathology of Alzheimer's disease is multifaceted with amyloid and tau aggregation, vascular changes and neuroinflammation (Malpetti et al., 2020a; Spires-Jones & Hyman, 2014). In this chapter, I assess the *in vivo* relationship between tau burden and synaptic density in the primary tauopathies of PSP and amyloid negative CBS cohorts. As reviewed in previous chapters and above, animal models of tauopathy have illustrated the colocalisation of misfolded tau protein and synaptic loss at the synaptic bouton (Hanger et al., 2019; Pooler et al., 2014) but the tau-synapse association is yet to be determined *in vivo*.

Figure 5-2 illustrates my hypotheses. Previous studies suggest that the strength of connectivity within a region and between brain regions can promote the spread of tau pathology, in humans (Cope et al., 2018) as in preclinical models (Ahmed et al., 2014). Therefore, I hypothesise that brain areas with higher synaptic density would develop more tau pathology. I predict that the spatial distribution of pathology, as measured with the PET radioligand [^{18}F]AV-1451, would be correlated with synaptic density, as measured with the PET radioligand [^{11}C]UCB-J. Since tauopathy in a region may impair efferent projections, a corollary hypothesis is that tau accumulation in one region (source region) leads to diaschisis characterised by reduced synaptic density in the areas to which it connects (target regions). I acknowledge the relatively low affinity of [^{18}F]AV-1451 for 4-repeat tauopathy compared to Alzheimer's disease, and the off-target binding of this ligand within the basal ganglia. I therefore refer to its binding target as 'molecular pathology', covering tau and

non-tau targets. A second part of the model describes the consequence of pathology, which is to reduce synaptic density. The predicted result is a positive relationship between [^{18}F]AV-1451 binding and synaptic loss, negatively moderated by disease progression.

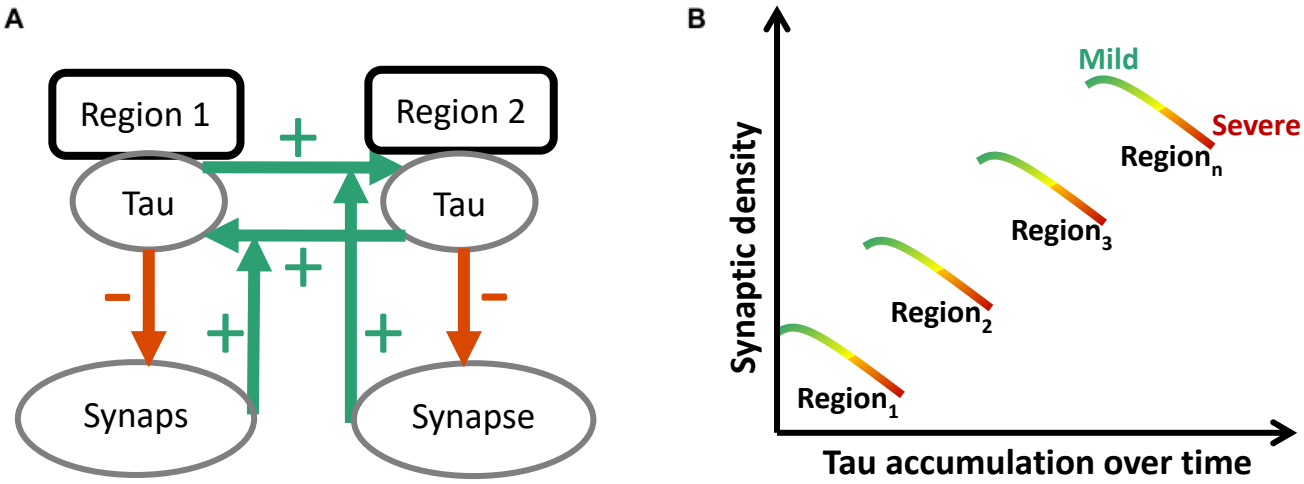

Figure 5-2. Schematic diagram illustrating the predicted toxic effect of tau on synaptic density.
At a regional level (A) synaptic density promotes the spread of tau from one region to another (for example from Region 1 to Region 2). Tau is however toxic to synapses, such that at a regional level it leads to a loss of synapses as the disease progresses. (B) Tau burden within a given region therefore, depends on a region's baseline synaptic density: for example, Region 3 with a high baseline synaptic density, would accumulate more tau in the mild stages of disease. But as the disease progresses over time, to moderate and advanced stages (yellow and red in B, respectively), with increasing tau accumulation, tau induced synapto-toxicity occurs with a decline in the number of synapses within any given region. The prediction would therefore be that, whilst in mild disease the degree of tau accumulation is dependent on baseline synaptic density, as the disease progresses this relationship breaks down, moving towards a negative direction between tau accumulation and synaptic density.

5.4 METHODS

5.4.1 PARTICIPANTS AND STUDY DESIGN

The current experiment and the experiments reported in Chapters 3 and 4 are part of the same study protocol. Thus, the participants, inclusion and exclusion criteria, and study procedure are as described in Chapter 2. In this chapter however, there are a few additional participants compared to Chapters 3-4; thus, the full sample available for assessment of the relationship between synaptic density and molecular pathology consisted of 19 controls, 12 patients with CBD, and 23 with PSP-Richardson's syndrome. Demographic details for this extended cohort are provided in Table 4-1. All participants underwent 3T MRI, [^{18}F]AV-1451 PET, and [^{11}C]UCB-J PET.

5.4.2 NEUROIMAGING PRE-PROCESSING

The procedure for [^{11}C]UCB-J synthesis, PET data acquisition, image reconstruction and kinetic analysis is as described in Chapter 2, and published in Holland et al. (2020). [^{18}F]AV-1451 synthesis and data acquisition followed the protocol described in Chapter 2.

5.4.3 STATISTICAL ANALYSES

Differences in demographic and clinical variables between the diagnostic groups were tested using ANCOVA, and chi-square tests where appropriate. A linear mixed effects model was used to assess the overall relationship between [^{18}F]AV-1451 and [^{11}C]UCB-J BP_{ND}, and the effect of group (patients vs controls) and brain region on this relationship. Subsequently, the patient data were normalised against controls by subtracting the mean regional control values (for both PET ligands) from each individual patient to remove any off-target binding beyond that seen in the basal ganglia. For this analysis, regions of interest with previously reported off-target binding of [^{18}F]AV-1451 (basal ganglia, and substantia nigra (Leuzy et al., 2019)) were excluded. To investigate the effect of individual variability on the relationship between [^{11}C]UCB-J and [^{18}F]AV-1451 BP_{ND}, a linear mixed effects model was used, allowing for an uncorrelated random slope and intercept per individual, with the interval between the two PET scans (in days) as a covariate of no interest. I subsequently extracted the slope of [^{11}C]UCB-J on [^{18}F]AV-1451 for each individual and used this in a linear model with the PSP rating scale (a measure of disease severity) as the

independent variable, and age as a covariate of no interest. To explore the correlation between [^{11}C]UCB-J and [^{18}F]AV-1451 BP_{ND} between regions, I calculated a correlation matrix between cortical [^{18}F]AV-1451 binding, and synaptic density in cortical and subcortical regions. Analyses were performed with and without partial volume correction, yielding similar results. I focus on partial volume corrected BP_{ND} to limit the potential effect of atrophy on our ligand cross-correlation but present data without partial volume correction in the supplementary material (Appendix C). Statistical analyses were implemented in R (version 3.6.2).

5.5 RESULTS

5.5.1 DEMOGRAPHICS

The patients (PSP and CBD) and control groups were similar in age, sex, education and injected activity of [^{11}C]UCB-J and [^{18}F]AV-1451 (Table 5-1). A typical cognitive profile for people with PSP and CBD was observed with impairment in verbal fluency, memory, and visuospatial domains of the ACE-R and MMSE.

	Control	PSP	CBD	F (p)
Gender (M:F)	11:8	10:13	7:5	ns[a]
Age at [^{11}C]UCB-J PET in years	68.9 (7.1)	71.3 (8.6)	70.9 (7.9)	ns
Symptom duration (years)	-	3.9 (2.2)	3.9 (2.1)	ns
Education (years)	13.6 (2.8)	12 (4.4)	12.5 (3)	ns
ACE-R total (max. 100)	96.7 (2.7)	81.5 (12.7)	81.2 (10.3)	8.8 (<0.001)
Attention_Orientation (max .18)	17.9 (0.3)	15.8 (4)	15.4 (5.2)	ns
Memory (max .26)	24.6 (1.7)	21 (6)	18.5 (8)	4.4 (0.02)
Fluency (max .14)	12.8 (1.0)	6.5 (3.3)	7.3 (3.6)	25.6 (<0.001)
Language (max .26)	25.6 (0.8)	22.2 (6.6)	20.5 (8.3)	3.3 (0.04)
Visuospatial (max .16)	15.7 (0.6)	12.3 (4.3)	12.2 (4.6)	5.7 (0.01)
MMSE (max. 30)	29.4 (1.2)	27.1 (2.6)	26.6 (3.0)	5.2 (0.01)
PSPRS (max. 100)	-	34 (9.4)	25.9 (12.4)	4.6 (0.04)
Injected activity (MBq)				
[^{11}C]UCB-J	370.7 (114.3)	322.2 (86.0)	320.4 (113.8)	ns
[^{18}F]AV-1451	182.3 (10.8)	182.1 (11.4)	186.1 (11.1)	ns
[^{11}C]UCB-J and [^{18}F]AV-1451 PET scan interval (in days)	157.2 (125.6)	155.9 (129.2)	45.5 (65.7)	4.6 (0.02)

Table 5-1. Demographics and neuropsychological profile for each participant cohort.
Results are given as mean (and standard deviation) unless otherwise stated. PSP refers to patients with PSP-Richardson's syndrome. CBD refers to amyloid negative corticobasal syndrome. The F-statistic and p-values are derived from ANOVA. ACE-R: revised Addenbrooke's Cognitive Examination, MMSE: Mini-mental State Examination, PSPRS: Progressive Supranuclear Palsy Rating Scale. [a] chi-squared test. ns = non-significant at $p<0.05$.

5.5.2 RELATIONSHIP BETWEEN [^{11}C]UCB-J BP_{ND} AND [^{18}F]AV-1451 BP_{ND}

In patients, there was a positive relationship between regional [^{18}F]AV-1451 BP_{ND} and [^{11}C]UCB-J BP_{ND} (β=0.3, t=4, p<0.0003). There was a significant region-by-[^{18}F]AV-1451 interaction (p<0.0001) driven by subregions of the frontal, parietal, temporal and occipital lobes, as well as the thalamus. There was no effect of PET scan interval on the overall significance of the model (p=0.7). There was individual variability in the slope of the relationship between [^{18}F]AV-1451 BP_{ND} and [^{11}C]UCB-J BP_{ND} (individual grey lines in Figure 5-3A). The direction of this relationship in each individual (i.e. the slope of each grey line in Figure 5-3A) negatively correlated with disease severity (β= -0.01, t= -2.2, R= -0.34, p=0.03), independent of age (p=0.4) (Figure 5-3B). In other words, those patients with more severe disease displayed a less positive relationship between [^{18}F]AV-1451 BP_{ND} and [^{11}C]UCB-J BP_{ND}. Similar findings were observed using BP_{ND} derived from data without partial volume correction (Figure C1).

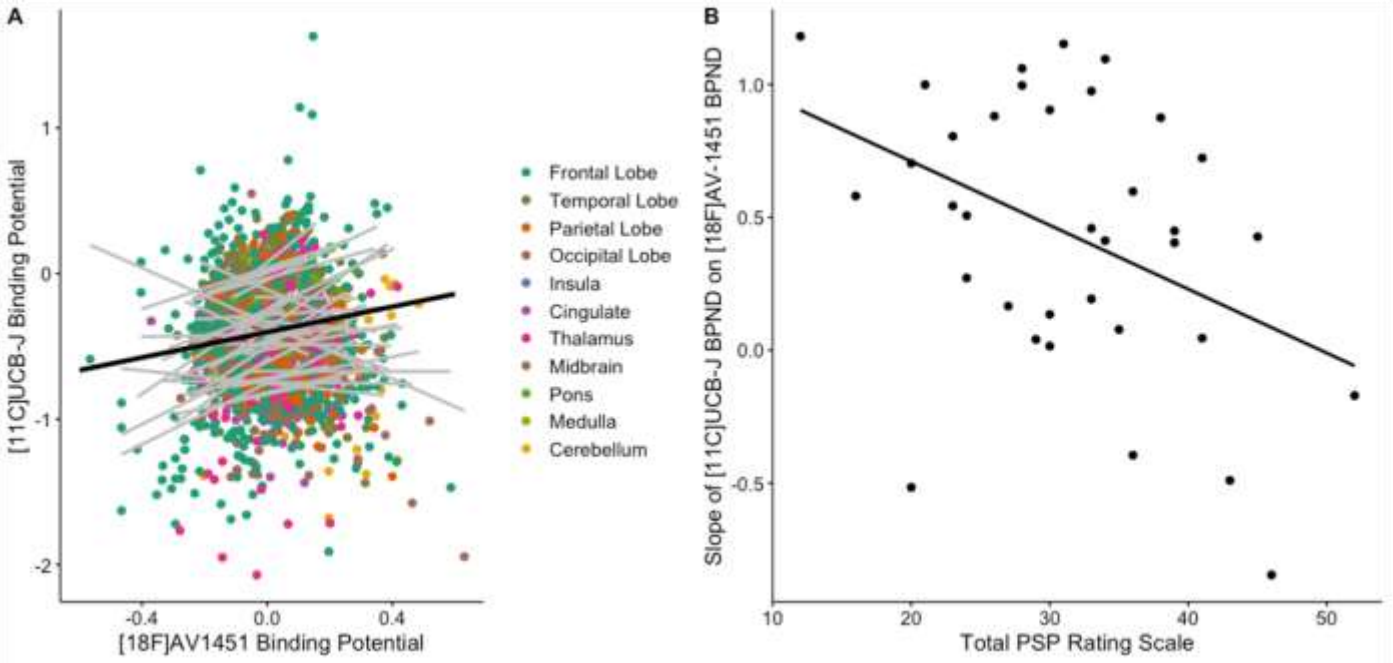

Figure 5-3. The association between synaptic density ([^{11}C]UCB-J) and molecular pathology ([^{18}F]AV-1451) is a function of disease severity.
A) Scatter plot of [^{11}C]UCB-J BP_{ND} and [^{18}F]AV-1451 BP_{ND} in 35 patients with PSP-Richardson's syndrome and amyloid-negative CBD (each grey line represents a patient) normalised against controls, across 73 regions of interest (excluding those with previously reported off-target binding, i.e. basal ganglia and substantia nigra); the dark black line in A depicts the overall fit of the linear mixed model, whilst grey lines represent individual patient participants. B) The slope for each individual (i.e. each grey line in A) is negatively correlated with disease severity (as measured with the PSP rating scale); R= -0.34, p<0.03.

5.5.3 CROSS-REGIONAL CORRELATION BETWEEN [^{18}F]AV-1451 BP_{ND} AND [^{11}C]UCB-J BP_{ND}

Synaptic density in a region is proposed to be affected by both local tau pathology and tau burden in connected regions from which it receives afferent projections. As a result, despite a positive correlation at a regional level, the synaptic density in any given region may be negatively affected by remote insult, with diaschisis between anatomically connected regions (illustrated schematically in Figure 5-2). As an exploratory analysis, the asymmetric Pearson's correlation matrix shown in Figure 5-4 was computed, between cortical [^{18}F]AV-1451 BP_{ND} (horizontal axis of matrix) and [^{11}C]UCB-J BP_{ND} (vertical axis of matrix) in cortical and subcortical regions in patients. Here I show that overall, there are significant negative correlations between cortical (frontal, temporal, parietal, and occipital) [^{18}F]AV-1451 BP_{ND} and subcortical [^{11}C]UCB-J BP_{ND} within the basal ganglia (nucleus accumbens, caudate nucleus and putamen). There were strong positive correlations between [^{18}F]AV-1451 BP_{ND} and [^{11}C]UCB-J BP_{ND} within the thalamus where strong local connections exist (Figure 5-4). Subcortical [^{18}F]AV-1451 BP_{ND} in the matrix in Figure 5-4 were not included, given the off-target binding in these regions which undermines the interpretability of the signal. However, I include these regions as well as other subregions in the larger correlation matrix in Supplementary Figure D3 for completeness. Similar findings are seen using BP_{ND} from data without partial volume correction (Supplementary Figure C2).

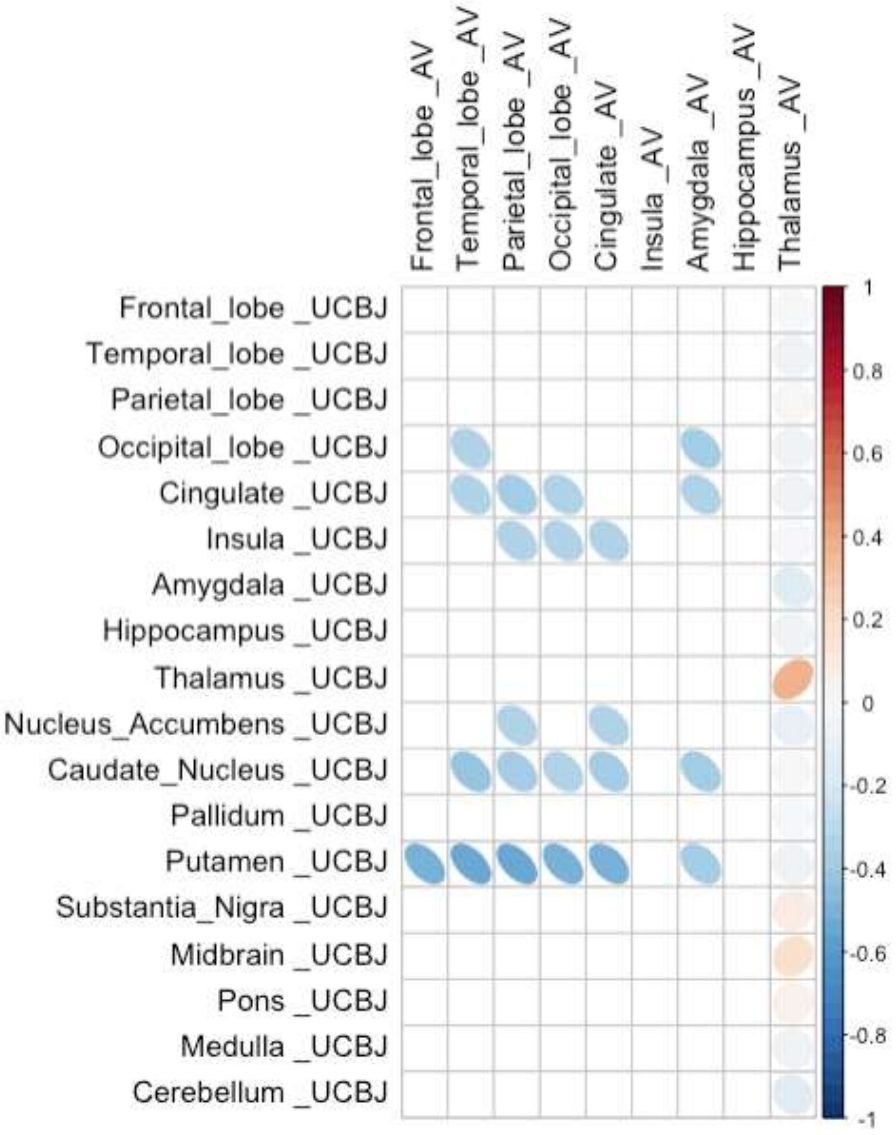

Figure 5-4. Cortical pathology is negatively correlated with subcortical synaptic density. Correlation between [^{18}F]AV-1451 BP_{ND} in cortical regions (horizontal axis) and [^{11}C]UCB-J BP_{ND} in a target region (vertical axis) both cortically and subcortically in patients. Only significant correlations (at $p<0.05$ uncorrected for multiple comparisons) are shown in this figure. The colour and width/shade of the ellipse depict the direction (i.e. blue = negative, red = positive correlations), and strength (i.e. narrow darker ellipses show stronger correlations), respectively.

5.6 DISCUSSION

In this chapter I have identified a complex relationship between molecular pathology (estimated with [^{18}F]AV-1451 PET) and synaptic density (estimated with [^{11}C]UCB-J PET), in patients with the primary tauopathies of Progressive Supranuclear Palsy and Corticobasal Degeneration (inferred *in vivo* from amyloid-negative corticobasal syndrome). There are three principal results: (i) regions with higher synaptic density have higher pathology, (ii) within regions, synaptic density becomes less dependent on [^{18}F]AV-1451 binding as disease severity increases, and (iii) between regions, increased cortical [^{18}F]AV-1451 binding is associated with reduced subcortical synaptic density. I interpret these three findings in the context of connectivity-based susceptibility to tauopathy, synaptotoxic effects of tauopathy, and cortico-subcortical diaschisis, respectively.

The effect of 4-repeat hyperphosphorylated tau pathology such as PSP and CBD, on synaptic function and density is complex. It involves both direct and indirect pathways of injury with changes in cellular physiology preceding the loss of neurons. Through direct pathways, pathological tau interferes with dendritic morphology, synaptic protein expression, the number of NMDA (N-methyl-D-Aspartate) and AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors on the presynaptic membrane, mitochondrial function, synaptic vesicle numbers, and ultimately synaptic loss (reviewed in Chapter 1 and in Jadhav et al. (2015)). Tau also directly affects the axon cytoskeleton and trafficking, as well as the soma (Kneynsberg et al., 2017). Indirectly, hyperphosphorylated tau adversely affects the functioning of the neuronal support network, including glia cells and astrocytes (Kovacs, 2020; Reid et al., 2020). These events are affected by the stage and severity of the disease process, and in relation to regional differences in connectivity which I discuss next (concepts schematically illustrated in Figure 5-2).

There was a positive relationship between the binding of [^{11}C]UCB-J and [^{18}F]AV-1451 such that areas of the brain with higher synaptic density develop higher pathology. This accords with preclinical and clinical models of tauopathy in which the strength of local network connectivity facilitates the transneuronal spread of tau pathology (Ahmed et al.,

2014; Clavaguera et al., 2013; Clavaguera et al., 2009; Cope et al., 2018; Polanco et al., 2018).

However, the relationship between tau accumulation and synaptic density changes with disease progression, at least as inferred from the cross-sectional moderation by disease severity. With increasing scores on the PSP rating scale, synaptic density becomes less dependent on local tau accumulation. In other words, in areas with relatively low tau accumulation synaptic density is minimally affected, whereas in areas with higher tau accumulation there is reduction of synaptic density as the disease progresses and this preferentially occurs in synapse rich areas. As the disease progresses, other pathological processes may contribute to synaptic loss, such as inflammation, another predictor of prognosis and mediator of synaptic loss (Malpetti et al., 2021). There is therefore not a simple linear relationship between tau accumulation and synaptic density in moderate and advanced disease. This observation accords with human post mortem and animal studies. In post mortem studies of the related tauopathy of Alzheimer's disease, there is a biphasic synaptic protein response during disease progression, with increases in synaptophysin/syntaxin/SNAP-25 in early Braak stages and synaptic loss observed only when the disease has progressed to the neocortex (Mukaetova-Ladinska et al., 2000). In the P301L transgenic mouse model of PSP-like tauopathy, there is a differential loss of synapses, as well as synaptic proteins, depending on disease stage (Kopeikina et al., 2013).

To understand the biphasic relationship between tau accumulation and synaptic density, one must consider other key players in synaptotoxicity in tauopathies, such as neuroinflammation (Palleis et al., 2020). Recent *in vivo* studies have confirmed the regional co-localisation of inflammation and [^{18}F]AV-1451 binding in PSP (Malpetti et al., 2020b), in line with previous *in vivo* (Gerhard et al., 2006; Gerhard et al., 2004), and post mortem reports of the tight interplay between neuroinflammation and tau accumulation in tauopathies (Kovacs, 2020). There is growing evidence that these two pathological processes affect synaptic function both independently and synergistically (Vogels et al., 2019).

The relationship between tauopathy and synaptic density is even more intriguing when considering the change in synaptic density in one region as a function of pathology in

another. There are strong correlations between [^{11}C]UCB-J binding within the basal ganglia and [^{18}F]AV-1451 binding in most cortical areas. The reverse association, between subcortical [^{18}F]AV-1451 and cortical [^{11}C]UCB-J binding is also observed (Supplementary Figure C3) but is dismissed here as uninterpretable in view of subcortical off-target binding of [^{18}F]AV-1451. The significant negative correlation between cortical [^{18}F]AV-1451 binding and synaptic density in the basal ganglia could be a reflection of severe disease in the basal ganglia and accumulating pathology in the neocortex. In other words, synapses are severely affected in the basal ganglia as one of the earliest sites of pathology, with pathology spreading and accumulating in synapse-rich areas of the brain, for example the neocortex. A second explanation is that loss of descending cortico-striatal axons due to cortical pathology, may cause diaschisis, affecting subcortical synaptic density even further. Previous reports of diffusion tensor imaging in patients with PSP/CBD have revealed extensive white matter abnormalities (within the main association fibres) beyond the degree of cortical atrophy (Borroni et al., 2008; Padovani et al., 2006) resulting in loss of cortical afferents onto subcortical structures. A third, though not mutually exclusive, explanation is the weakening of cortical-subcortical functional connectivity resulting from dysfunctional synapses rather than synaptic loss as suggested from previous functional connectivity studies in PSP (Cope et al., 2018; Gardner et al., 2013).

Although at a regional level there is a positive correlation between [^{11}C]UCB-J and [^{18}F]AV-1451 BP_{ND}, synaptic function or synaptotoxic tau oligomers are not being directly measured here. This caveat must be borne in mind when interpreting PET data. It is the preclinical models that have shown that oligomers of tau are toxic to synaptic function, even in the absence of tau polymers/fibrils (Acquarone et al., 2019; Fá et al., 2016; Kaniyappan et al., 2017; Menkes-Caspi et al., 2015; Niewiadomska et al., 2021). By the time tau aggregates are established, oligomers of tau are expected cortically, and perhaps interfering with cortical function and the integrity of descending axons.

There are a few limitations of this experimental chapter. First, the low affinity of [^{18}F]AV-1451 for PSP and CBD 4-repeat tau. Even where this radioligand recapitulates the distribution of post mortem neuropathology in PSP and CBD, and binds PSP 4-repeat tau, the affinity is very much lower than for 3-repeat tau in Alzheimer's disease (Soleimani-Meigooni et al., 2020; Y. T. Wang & Edison, 2019). Second, there is well-established off-

target binding of [^{18}F]AV-1451, particularly within subcortical structures where monoamine oxidase is present. Off-target binding is most prominent in the basal ganglia which I excluded before running the statistical analysis in this chapter, in addition to normalising the patient data against controls. I included these regions in the detailed descriptive correlation matrices in Supplementary Figure C2 and 3 for completeness sake, noting the strong negative correlations between cortical [^{18}F]AV-1451 BP_{ND} and subcortical [^{11}C]UCB-J $BP_{ND.}$ Third, I note that in PET studies of neurodegeneration with atrophy, grey matter volume loss can affect the interpretation of PET signals. However, synaptic loss in PSP and CBD occurs even in areas of the brain without discernible atrophy on MRI as shown in Figure 3-4 and Figure 4-3. Nevertheless, a stringent partial volume correction method (GTM) has been used, to minimise the effect of atrophy on the ligand cross-correlations. Of note, the data without partial volume correction yield similar results in all the main analyses (Supplementary Figure C1 and 2). Lastly, the cross-sectional design of this study limits the interpretation of the dynamic relationship between tau accumulation and synaptic loss. Although I include patients at various stages of their illness, a longitudinal design is necessary to test the proposed dynamic relationship, and the mediation of synaptic loss by progressive tauopathy.

In conclusion, I have demonstrate a widespread positive association between [^{18}F]AV-1451 and [^{11}C]UCB-J binding in patients with symptomatic PSP and amyloid-negative corticobasal syndromes. Individual variability in this association correlates with disease severity. The complex relationship between tau accumulation and synaptic density *in vivo* may explain changes in cognitive and motor physiology. I hope that these insights will inform the design of new clinical trials to arrest PSP and CBD.

6 GENERAL DISCUSSION

Summary

The final chapter of this thesis brings together the hypotheses and evidence presented in Chapters 3 to 5, summarising the key findings, and emphasising how they advance our understanding of synaptic pathology in neurodegeneration. I then integrate this work with the prior literature, discuss significant limitations, and provide directions for future research. Specifically, I discuss (i) the key role of synaptic pathology as an early pathological mechanism in primary tauopathies and other neurodegenerative disorders, and (ii) therapeutic options for the maintenance and recovery of synapses in neurodegeneration.

6.1 REVIEW OF THE PRINCIPAL FINDINGS

In the introduction to this thesis, I proposed that synaptic loss is critical to the pathogenesis of primary tauopathies. In chapter 3, I tested the specific hypothesis that synaptic density is reduced by the primary tauopathies of Progressive Supranuclear Palsy (including Richardson's syndrome, PSP-RS) and amyloid-negative corticobasal syndrome (interpreted as due to CBD), and that this reduction correlates with disease severity. A new cohort of patients with PSP and CBD, as well as healthy controls underwent synaptic PET imaging with [^{11}C]UCB-J, structural MRI imaging, as well as neuropsychological assessment. A general linear model assessed group differences in synaptic density (patients versus controls), and relationship between synaptic density and disease severity. I confirmed a significant loss of synapses in the patient cohort, even in areas of the brain without significant volume loss. I illustrated that synaptic loss was highly correlated with functionally relevant measures of cognition, and with disease severity. The results are the first to demonstrate *in vivo* synaptic loss in primary tauopathies; they enable better understanding of the early pathophysiology of these neurodegenerative diseases, with outcomes directly relevant to the design of clinical trials aiming to restore or maintain synapses.

Having observed presynaptic synaptic loss, in chapter 4, I tested whether loss of the presynaptic compartment relates to changes to the postsynaptic compartment, in terms of loss of dendritic complexity as measured with the Orientation Dispersion Imaging (ODI) with MRI. I confirmed that in both PSP and CBD, there is widespread reduction in ODI, which is tightly linked to the loss of presynaptic SV2A (in presynaptic vesicles). The ODI results offer the possibility of utilising less invasive methods as complementary and a surrogate marker of synaptic density in future clinical studies.

A third question addressed in this thesis, was how changes in synaptic density relate to pathology in PSP and CBD. In chapter 5, I show a complex correlation between synaptic loss and pathology, mediated by disease severity. Furthermore, I report a negative correlation between molecular pathology in one region and synaptic densities in areas of the brain that are structurally and functionally connected with it.

The findings here are in accord with other neurodegenerative disorders, and suggest synaptic toxicity is a common pathogenic feature, offering a potential target for new therapeutic strategies.

6.2 Synaptic loss in Primary Tauopathies

The *in vivo* loss of synapses in corticobasal degeneration (CBD) and Progressive Supranuclear Palsy-Richardson's syndrome (PSP) is significant and widespread (chapter 3). It is seen in the brain areas identified as those affected early in the disease (for example basal ganglia (Kovacs et al., 2020)), and areas associated with minimal volume loss (for example frontal cortical regions). This latter finding suggests synaptic pathology in some areas begins before the large scale cell death, and may be as result of toxic oligomeric tau, as evident from animal models of tauopathy (Fá et al., 2016; Kaniyappan et al., 2017; Niewiadomska et al., 2021). The synaptic loss is potentially identifiable in both the pre- and postsynaptic compartments (chapter 4). This is suggested *in vivo* in PSP and CBD, through the tight coupling between [^{11}C]UCB-J binding (presynaptic density), and the Orientation Dispersion Index (ODI; measuring postsynaptic dendritic complexity). However, the presynaptic change in both patient groups had a higher effect size than changes in ODI (Cohen's d, Figure 4-3C and Figure 4-4C). This observation may in part, be due to [^{11}C]UCB-J being a more specific and accurate surrogate for presynaptic density as it directly binds a presynaptic vesicle protein (Finnema et al., 2016), than ODI is for postsynaptic complexity, where it measures structural integrity and complexity rather than specific postsynaptic proteins (H. Zhang et al., 2012). Nevertheless, the significant reduction in presynaptic density, and dendritic complexity is in accordance with animal models of tauopathy (Clare et al., 2010; Herms & Dorostkar, 2016; Tyebji & Hannan, 2017) and *in vivo* studies of other neurodegenerative disorders where synaptic and dendritic pathologies are interlinked and key pathogenic mechanisms (M. K. Chen et al., 2018; Matuskey et al., 2020; Mecca et al., 2020; Nicastro et al., 2020).

Synapses play a central role in memory formation and cognition (Stewart et al., 2014; Südhof, 2004). I recapitulate this in PSP and CBD (chapter 3), where a strong correlation between *in vivo* global synaptic density, and global cognition and disease severity is observed (Figure 3-5). This close correlation between *in vivo* synaptic density and cognition has been replicated in the closely related tauopathy of Alzheimer's disease, where reduced

hippocampal binding of the synaptic PET ligand [^{11}C]UCB-J is associated with lower episodic memory and higher scores on the clinical dementia rating scale (Mecca et al., 2020).

Tau-induced synaptic toxicity is well documented in animal models, acting through direct and indirect pathogenic mechanisms (chapter 5). In chapter 5, I illustrate that the binding of [^{11}C]UCB-J colocalises, and is strongly positively correlated, with that of [^{18}F]AV-1451, suggesting that areas of the brain with a higher synaptic density, harbour more pathology. Although the interpretation of the signal from [^{18}F]AV-1451 PET in 4-repeat tauopathies is currently under debate (chapter 5), the *in vivo* colocalisation of the two measures is in line with animal and post mortem studies (Spires-Jones & Hyman, 2014; Vogels et al., 2019). This positive correlation between synaptic density and the underlying pathology has also been observed in Alzheimer's disease where, amyloid PET ligand binding is positively associated with *in vivo* hippocampal synaptic density (O'Dell et al., 2021). In PSP and CBD, the positive correlation between synaptic density and molecular pathology is moderated by disease severity (Figure 5-3B), similar to findings in a recent [^{11}C]UCB-J PET study in Alzheimer's disease; Coomans *et* al. report that the *in vivo* relationship between cortical tau PET binding and synaptic density is moderated by each individual's cortical tau burden, such that with higher tau load, the relationship becomes negative (Coomans et al., 2021).

Although connectivity is not directly measured in Chapter 5, it is central to the interpretation of the results. For example, despite synaptic loss in PSP and CBD, it is possible that the functional connectivity of the remaining synapses is strengthened as a compensatory mechanism, therefore promoting accrual of pathology locally (at least in the early stages of disease). Indeed, increased cortical-cortical functional connectivity, as measured with resting-state function MRI, in PSP and CBD has been previously reported (Cope et al., 2018; Filippi et al., 2019). Long-distance functional connections (for example cortical-subcortical connections) are however weakened in primary tauopathies (Filippi et al., 2019; Gardner et al., 2013) providing a potential explanation for the negative correlations observed between cortical pathology and subcortical synaptic density Figure 5-4.

Synaptic pathology is not unique to the primary tauopathies of PSP and CBD. Indeed, it is a central pathogenic mechanism underlying the cognitive deficits, and clinical phenotypes

of many neurodegenerative diseases, as well as a feature of the neuropsychiatric disorders depression and schizophrenia. Together these disorders place a considerable social, emotional, and financial burden on sufferers, care providers, and health services. There is therefore an urgent need to develop therapeutic strategies to delay or halt progression of this group of conditions. Given the critical role synapses play in the pathogenesis, they provide the perfect platform for the development of synapse-specific therapeutics with a much broader application alongside disease-specific treatment advances. In the following section, I will review the central aspects of synaptic dysfunction in prevalent neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease and Lewy body dementia, frontotemporal lobe dementia, and prion disease.

6.3 SYNAPTIC LOSS IN OTHER NEURODEGENERATIVE DISORDERS

Synapses are susceptible to a range of neurodegenerative stimuli, from trauma to infectious agents to underlying genetic risk factors. Given their key role in learning and memory (reviewed in Kandel et al. (2014)), any persistent perturbations in their normal physiology, as seen in neurodegeneration or chronic psychiatric disorders, result in malfunction and degeneration contributing to clinical symptoms. The increasingly used term 'synaptopathy' refers to the group of disorders where the above process occurs early and is a central pathogenic mechanism – I review these below:

6.3.1 ALZHEIMER'S DISEASE

Alzheimer's disease (AD) is the commonest cause of neurodegenerative dementia (accounting for 60% of cases (Ferri et al., 2005)) and a leading cause of global disease burden. It is characterised pathologically by abnormal tau accumulation in neurofibrillary tangles (mixed 3 and 4 repeat) and the presence of extracellular amyloid-beta deposition (Selkoe & Hardy, 2016); clinically it presents with progressive memory loss and behavioural changes (Montine et al., 2012). The pathological hallmarks of AD however, do not correlate well with the severity of dementia or neuronal loss (Kril et al., 2002), unlike synaptic loss which occurs early in the pathogenesis (Scheff et al., 2007), is closely associated with cognitive impairment (Scheff et al., 2006; Terry et al., 1991), and directly related to the duration of dementia (Blennow et al., 1996; Ingelsson et al., 2004)

There is a wealth of evidence for synaptic loss in AD from animal models, post mortem data (reviewed in Honer (2003) and De Wilde et al. (2016)), and recent *in vivo* studies using the presynaptic ligand [^{11}C]UCB-J or closely related analogues of this (M. K. Chen et al., 2018; Coomans et al., 2021; Mecca et al., 2020; O'Dell et al., 2021). Significant reductions in synaptic density are seen in the hippocampus, fronto-temporal cortices, the cingulum and the entorhinal cortex, with the earliest and the most significant alterations in presynaptic proteins, seen within the hippocampus. The ubiquitous presynaptic protein synaptophysin is widely studied in neurodegeneration, but the extent of synaptic damage is more widespread and involves many other key proteins, albeit to varying degrees. Some key affected proteins in AD are those involved in the SNARE (soluble NSF attachment protein receptor) machinery including: SNAP-25, VAMP and syntaxin, key to the fusion of synaptic vesicles with the presynaptic membrane and neurotransmitter release. Other affected presynaptic proteins include the Rab family, calcium sensory proteins, and those key to the neuronal cytoskeleton. Likewise at the postsynaptic membrane, reductions in synaptic proteins responsible for the postsynaptic scaffolding and dendritic structure, as well as glutamate receptors and mitochondrial proteins, are seen. Additionally, amyloid beta oligomers engage synaptic receptors (Fyn, CDK5, and GSKβ) that trigger neurotoxic signalling pathways, leading to neuronal autophagy (reviewed in Honer (2003) and De Wilde et al. (2016)).

Whilst both tau tangles and amyloid plaques are known to cause neuronal loss, the degree to which the latter occurs exceeds that of tau tangles suggesting that tangles are not necessary for neuronal death in AD (Gómez-Isla et al., 1997). The toxic pathways leading to synaptic dysfunction and loss are in part due to the direct and indirect deleterious effects posed by both pathological tau and beta amyloid at the synapse (Spires-Jones & Hyman, 2014). However, whilst the loss of some synaptic proteins is directly linked to neurofibrillary tangles, for example synaptophysin (Callahan & Coleman, 1995), the loss of others such as Rab3A is not (Blennow et al., 1996). Indeed, it is tau oligomers that are responsible for the majority of the toxic damage caused at the synapse both directly (Menkes-Caspi et al., 2015), and also indirectly by interfering with microtubule assembly, neuronal cytoskeleton, axonal transport and the integrity of the nucleus membrane, thereby jeopardising the DNA transcription and translation machinery (reviewed in (Niewiadomska et al., 2021)). Similarly, synaptophysin immunoreactivity in mouse models of AD, show

an intact signal in response to amyloid beta fibrils and monomers (Ishibashi et al., 2006), but with a significant reduction in response to amyloid beta oligomers, where the latter is found to be a predictor of synaptic change in the entorhinal cortex and super frontal gyrus (Lue et al., 1999). These studies suggest that, as well as in their fibrillar and monomeric forms, amyloid beta and pathogenic tau in their soluble and oligomeric forms are especially toxic to synapses similar to observations seen in the P301L mouse model of primary tauopathy (Menkes-Caspi et al., 2015). Recent *in vivo* PET studies in AD, confirm AD region specific synaptic loss colocalising with PET radioligands targeting amyloid plaques (O'Dell et al., 2021) and neurofibrillary tangles (Coomans et al., 2021); these observations are in line with preclinical data, however we are as yet unable to image oligomers *in vivo* where the association with synaptic loss may be greater, echoing *in vitro* findings.

6.3.2 FRONTOTEMPORAL DEMENTIA

Frontotemporal dementia (FTD) is a clinical syndrome characterised by changes in personality, behaviour, and language (Gorno-Tempini et al., 2011; Rascovsky et al., 2007). It was formerly known clinically as Pick's disease, although this term is now generally reserved for the particular pathological entity with 3-repeat tau aggregation linked to mutations in the microtubule-assisted tau protein (MAPT) gene (Irwin et al., 2016). Other causes of frontotemporal dementia include TDP-43 inclusions or fused-in-sarcoma (FUS) pathology starting in but not limited to the frontal and temporal lobes and extending to include the rest of the brain with disease progression (Rohrer et al., 2011). FTD falls within the spectrum of frontotemporal lobar degeneration syndromes (Figure 1-2), sharing phenotypic and pathological features with PSP and CBD (Chapter 1).

Genetically, hexanucleotide repeat expansions of the chromosome 9 open reading frame 72 (C9orf72), and mutations in the granulin, TAR DNA-binding protein (TDP43), fused-in sarcoma (FUS), and MAPT gene may cause either a behavioural variant FTD syndrome, motor neuron disease (MND), mixture of FTD-MND, or FTD and parkinsonism linked to chromosome 17 (Mackenzie et al., 2011; Rohrer et al., 2015). Reductions in synaptophysin immunochemistry in FTD was first reported in 1995, in a regionally specific manner including the superficial layers of the prefrontal cortex but not in parietal, inferior temporal or posterior regions (Brun et al., 1995; Liu & Brun, 1996). Since then, reductions in other synaptic vesicle proteins Rab3A, synaptotagmin, synapsin-1, and presynaptic plasma

membrane proteins SNAP-25 and syntaxin-1, within the upper but not deeper layers of the frontal cortex have been reported, as well as reduced CSF synaptic marker neuronal pentraxin-2 (van der Ende et al., 2020). The postsynaptic compartment in FTD is also affected where marked reductions in dendritic spine numbers in the apical dendrites of pyramidal neurons are seen (I. Ferrer, 1999). Using a more sensitive protein staining and quantification method such as ELISA (enzyme-linked immunosorbent assay), Lipton *et* al. have reported a more widespread loss of synaptic proteins extending beyond the frontal lobes, into the tempro-parietal lobes, albeit in a small sample of post mortem brains of patients with FTD (Lipton et al., 2001).

Animal models of primary tauopathies, such as the P301S (Hoffmann et al., 2014) or rTG4510 models (Rocher et al., 2010), harbour human MAPT gene mutations responsible for some phenotypes of frontotemporal lobe dementia and PSP-like disorders. Both these models, feature mutated tau oligomers and tangles, present with synaptic dysfunction and dendritic pathology in the prefrontal cortex and the hippocampus, and show difficulties in learning and memory (Kaniyappan et al., 2017; Liu & Brun, 1996; Menkes-Caspi et al., 2015; Schindowski et al., 2006).

Other protein aggregates associated with FTD, TDP-43, FUS, ubiquitin and p62 positive inclusions, have all been observed within dendritic spines (Brettschneider et al., 2012). In the transgenic mouse model harbouring the ubiquilin-2 mutation seen in some patients with FTD ($UBQLN2P^{497H}$), ubiquitin and p62 positive inclusions within dendrites are associated with dendritic spine pathology, synaptic dysfunction, and cognitive deficits (Gorrie et al., 2014).

6.3.3 Parkinson's Disease and Lewy Body Dementia

Parkinson's disease (PD) is the second most common neurodegenerative disease, characterised pathologically by the presence of intraneuronal alpha-synuclein containing Lewy bodies (Spillantini et al., 1997). Clinically it presents with a characteristic movement disorder typically manifesting as bradykinesia, rigidity, tremor and postural instability (Postuma et al., 2015), and later in the disease course cognitive decline (Williams-Gray et al., 2009). Lewy body pathology, the hallmark of PD, occurs predominantly in the substantia nigra *pars compacta* but it is also found in the locus coeruleus and is associated with cell loss (Halliday et al., 1990). As with other neurodegenerative disorders, synaptic

protein reductions in PD are observed in post mortem brains of patients, in particular reductions in the synaptosomal-associated protein 25 (SNAP25) which provides the driving force for synaptic vesicle fusion, and docking (Bereczki et al., 2016). Synaptic protein dysfunction and reductions in PD are as a result of direct pathological aggregates of alpha-synuclein but also the downstream effect of dysfunctional proteins from PD-linked genetic mutations.

Synaptic dysfunction is a key pathogenic mechanism in PD in two respects (reviewed in Picconi et al. (2012), Pienaar et al. (2012), Nguyen et al. (2019), and Koziorowski et al. (2021)). First, alpha-synuclein (encoded by the SNCA gene) normally resides in the presynaptic compartment, promoting SNARE-complex assembly (Burré et al., 2010), and thus central to the regulation of synaptic activity, plasticity, synaptic vesicle pool maintenance and trafficking. Mutations in the synuclein protein and the subsequent aggregation in the presynaptic compartment, redistributes proteins of the presynaptic SNAR complex, and therefore interferes with synaptic vesicle fusion, and exo- and endocytosis (Bridi & Hirth, 2018; Garcia-Reitböck et al., 2010; Yavich et al., 2004), manifesting as cognitive dysfunction (Howlett et al., 2015).

Second, PD-related genetic mutations in LRRK2, DJ1, PINK1, and PRKN genes, are deleterious to the harmonious interaction between synaptic vesicle proteins (including SV2A) involved in synaptic vesicle transport (Abeliovich & Gitler, 2016; Belluzzi et al., 2012; Plowey & Chu, 2011). Mutations in the above genes therefore reduce synaptic vesicle trafficking and neurotransmitter release (Esposito et al., 2012). Other recently identified PD-linked genes involved in synaptic endocytosis are also thought to contribute to PD pathogenesis. Such genes include DNAJC6 (encoding auxilin (Olgiati et al., 2016)), SYNJ1 (encoding synaptojanin-1 (Quadri et al., 2013)), and SH3GL2 (encoding endophilin A1 (Chang et al., 2017)). Similar to PSP pathogenesis, LRRK2 mutations play a particular role in PD pathogenesis by interfering with the functioning of other PD-linked gene products. For example, in a drosophila model of PD, LRRK2 mutations lead to abnormal phosphorylation of endophilin A1, and therefore interferes with its interaction with synaptotagmin-1 (Islam et al., 2016), and in mouse models lead to reductions in synaptic vesicle density (Xiong et al., 2018).

Observations from animal models and post mortem studies have recently been confirmed *in vivo* with UCB-J PET, where reduced binding are reported in subcortical areas (substantia nigra, caudate, locus coeruleus), as well as in the cingulate and the prefrontal cortex (Andersen et al., 2021; Matuskey et al., 2020). Of note, no differences in volumetric measures were observed between patients and controls despite the significant loss of synapses; this is in line with preclinical data where synaptic dysfunction is seen before overt neuronal loss (Janezic et al., 2013). Although Matuskey *et* al. did not observe a correlation with clinical measures (possibly due to a small sample size and absence of participants with a clinical diagnosis of PD dementia), Andersen *et* al report a positive correlation between synaptic density and executive cognitive domains in patients with Parkinson's dementia (Andersen et al., 2021).

Similar to Parkinson's disease, Lewy Body Dementia (LBD) is a neurodegenerative disorder associated with an underlying alpha-synucleinopathy. Clinically it is characterised by parkinsonism, early cognitive dysfunction, recurrent visual hallucinations, and rapid eye movement sleep behaviour disorder (McKeith et al., 2017). Despite a more aggressive form of cognitive dysfunction (Oesterhus et al., 2014; Williams et al., 2006), brain atrophy is less pronounced in LBD compared to AD (Mak et al., 2015). Synaptic pathology has therefore been proposed as a central pathogenic mechanism. Indeed, animal models of LBD (Kramer & Schulz-Schaeffer, 2007), and post mortem data (Bereczki et al., 2016) confirm presynaptic protein and dendritic spine loss, contributing to cognitive dysfunction. Bereczki *et* al. report a significant reduction in the concentration of neurogranin (a postsynaptic protein involved in the regulation of synaptic transmission through binding to calmodulin), the presynaptic vesicle protein Rab3A (reflective of the recycling pool of synaptic vesicles), and SNAP-25, with the latter two correlating with cognitive decline (Bereczki et al., 2016). Recent *in vivo* [^{11}C]UCB-J PET studies have reported reduced synaptic density within the substantia nigra and cortical regions (Andersen et al., 2021; Nicastro et al., 2020), correlating with clinical measures (Andersen et al., 2021) in the absence of overt atrophy.

6.3.4 HUNTINGTON'S DISEASE

Huntington's disease (HD) is a neurodegenerative disorder caused by a mutation in the huntingtin gene causing a trinucleotide repeat expansion, presenting with a movement

disorder and cognitive dysfunction (F. O. Walker, 2007). HD provides an opportunity to study the early preclinical phase of neurodegeneration in presymptomatic individuals carrying the culprit mutation, and also in mouse models where copy numbers of the trinucleotide expansion can be controlled giving rise to a spectrum of clinical severity. The key pathological hallmark of HD is the degeneration of the striatum with selective vulnerability of medium-spiny neurons which receive significant cortical projections (de la Monte et al., 1988); under normal physiology these cortico-striatal projections are rich in the protein huntingtin (Fusco et al., 1999). Abnormal functioning of these projections are evident even in the preclinical phase where neuronal loss is absent (reviewed in Cepeda et al. (2007) and in Levine et al. (2004)), suggesting that synaptic pathology plays a key role in the early pathogenesis of HD. In transgenic HD mouse models (e.g. R6/2), and in patients with HD, the concentration of complexin II (a protein that regulates fusion between synaptic vesicles and the presynaptic membrane) is reduced in neurons expressing mutant huntingtin (Morton & Edwardson, 2001). Similarly, the phosphorylation of synapsin 1 (a presynaptic protein that attaches to the cytoskeleton and involved in vesicle exocytosis), is also significantly reduced in neurons expressing mutant huntingtin (Xu et al., 2013). Postsynaptically, mutant huntingtin expressed in the R6/1 transgenic mouse model, results in reductions in PSD-95 (a membrane associated kinase, acting as the major scaffolding protein involved in dendritic spine changes and synaptic plasticity) (Nithianantharajah & Hannan, 2013).

At the synapse, under normal physiology, huntingtin interacts with vesicular structures such as clathrin-coated vesicles, endosomal compartments, and microtubules (Velier et al., 1998), and in its mutant form therefore, disrupts neurotransmitter synthesis, transport and release (reviewed in Tyebji and Hannan (2017)). At the postsynaptic membrane, clathrin, via its adaptor protein 2 (AP2), mediates neurotransmitter receptor surface expression. AP-2, along with huntingtin and huntingtin-interacting protein-1, bind to AMPA and NMDA receptor for internalisation (Metzler et al., 2007); mutant huntingtin disturbs this physiological interaction and thus interferes with signal transduction between the pre- and postsynaptic compartments (Milnerwood et al., 2010).

Transgenic mouse models of HD harbouring mutant huntingtin, exhibit changes in membrane potential leading to changes in synaptic input (Stern, 2011). The latter leads to an imbalance in inhibitory and excitatory inputs (Spampanato et al., 2008) thus causing

alterations in long-term depression and long-term potentiation and short-term plasticity in the frontotemporal cortices (Cummings et al., 2007; Dallérac et al., 2011), reflective of the cognitive impairment seen clinically.

An additional deleterious effect of mutant huntingtin is in inducing alterations in the activity of the brain derived neurotrophic factor (BDNF), and its transport along the axon. BDNF has a central role in synaptic plasticity and synaptic health (reviewed in Tyebji and Hannan (2017)) by interacting with other key proteins and regulating intracellular calcium stores, but also through inducing change in neurite morphology and spine density (Herms & Dorostkar, 2016). This pathogenic mechanism has been of interest therapeutically. For example, in mouse models, treatment with long-term BDNF increases spine density in the hippocampal CA1 region (Tyler & Pozzo-Miller, 2001), and enlarges dendritic spines at glutamatergic synapses (Tanaka et al., 2008), offering a potential therapeutic avenue for synaptic restoration.

6.3.5 PRION DISEASE

Prion disease is caused by the misfolding of the prion protein, clinically manifesting as a rapidly progressive and fatal dementia with motor and psychiatric symptoms (Aguzzi et al., 2007). Post mortem brains of patients with prion disease, and mice transfected with the mutant prion protein show reduced synaptic density (reviewed in Senatore et al. (2013)); specifically, reduced synaptophysin, synapsin-1, syntaxin-1, SNAP-25, and PSD-95 (I. Ferrer, 1999, 2002). Mice transfected with the mutated prion protein show abnormal endoplasmic-reticulum dependent phosphorylation of key synaptic proteins, leading to degeneration of the presynaptic compartment and loss of dendritic spines in the postsynaptic compartment, preceding cell death (Jeffrey et al., 2000). This toxic effect of misfolded prion protein is thought to occur in response to large aggregates but also in response to monomers and soluble oligomers similar to observations in primary tauopathies (Simoneau et al., 2007; Zhou et al., 2012).

6.3.6 NEUROPSYCHIATRIC DISORDERS

Synaptic dysfunction forms a key part of neuropsychiatric disorders including autism spectrum disorders, schizophrenia and depression. Given the heterogeneity of neuropsychiatric disorders, it is suggested that mapping transdiagnostic symptoms to their underlying neurobiology and brain circuitry may have a greater translational impact than

identifying targets for categorical disorders (Cuthbert, 2014). One such common neurobiological pathway is synaptic pathology. For example, persistent high levels of stress is thought to result in synaptic loss in circuits underlying affective and cognitive disorders (Duman et al., 2016), potentially contributing to the symptoms of depression. Post mortem studies in patients with major depressive disorders show reduced synaptic density in the dorsolateral prefrontal cortex (Kang et al., 2012), consistent with *in vivo* evidence of network disruption in the prefrontal cortex and connected limbic regions (Kaiser et al., 2015; Murrough et al., 2016). Using [^{11}C]UCB-J PET imaging, Holmes *et* al. have recently shown reduced synaptic density in the anterior cingulate, hippocampus, and dorsolateral prefrontal cortex, in patients with severe depression in accordance with post mortem data. They further show a negative correlation between prefrontal synaptic density and performance on working memory in line with previous reports in this condition (S. E. Holmes et al., 2019; Johnsen & Asbjørnsen, 2008). Chronic neuroinflammation has been identified as a major pathological mechanism underlying depression. Given the key role astrocytes play in synaptic maintenance, it is perhaps not surprising to see synaptic loss in response to a chronic state of neuroinflammation, and synaptotoxic chemical release (reviewed in Troubat et al. (2021)), similar to the synapto-toxic effects of neuroinflammation in PSP/CBD (Kovacs, 2020; Malpetti et al., 2020b).

Synaptic dysfunction and loss has been identified as a central pathogenic mechanism in schizophrenia – a neurodevelopmental psychiatric illness (much like autism spectrum and bipolar disorders). Schizophrenia presents with positive (delusions, hallucinations, and personality change) and negative symptoms (apathy, and withdrawal) (McCutcheon et al., 2020), and is associated with abnormal synaptic pruning and connections (Germann et al., 2021). Post mortem data illustrates reduced synaptic proteins (synaptic vesicle proteins, synaptophysin , synaptobrevin, PSD-95) and dendritic spine density (Osimo et al., 2019) in the frontal cortex and the anterior cingulate, in line with reduced *in vivo* [^{11}C]UCB-J PET signal in patients (Onwordi et al., 2020), and *in vivo* altered functional connectivity (Mwansisya et al., 2017).

In summary, the pathways leading to synaptic toxicity as a result of toxic species of disease-specific protein aggregates, can affect the synapse either by interfering with the surrounding environment (e.g. mitochondrial dysfunction, and neuroinflammation) or by disturbing synaptic protein homeostasis (as discussed above). The two pathways are not mutually

exclusive and occur in parallel with the end result of overwhelming neuronal stress, abnormal signal transmission, and weakening of functional connectivity. Common to the pathogenesis of the neurodegenerative disorders explored above, is the disturbance in synaptic function, stability and integrity, before structural breakdown. Therefore, subtle changes in functional processes within the synaptic compartments (for example, imbalances in synaptic proteostasis) have wide ranging and profound implications on synaptic plasticity, and connectivity. Synaptic manipulation, restoration and maintenance therefore provides a therapeutic platform early in neurodegeneration, before the onset of overt function impairment, as depicted schematically in Figure 1-3.

6.4 TARGETING THE SYNAPSE THERAPEUTICALLY

The maintenance, compensation and recovery of synapses in neurodegenerative processes may provide potential therapeutic avenues early in neurodegeneration (reviewed in J. Jackson et al. (2019)).

Early therapeutic approaches towards synaptic function have focused on synaptic maintenance by attempting to improve the efficiency of remaining synapses and boosting the levels of deficient neurotransmitters; for example by using the following pharmacological agents: anti-cholinesterase inhibitors (donepezil, rivastigmine) (Anand & Singh, 2013) and NMDA receptor antagonist memantine in mild to moderate Alzheimer's disease (Prentice et al., 2015); dopaminergic therapy for Parkinson's disease and Lewy Body dementia (Mao et al., 2020), PSP and CBD (albeit with a milder benefit compared to PD) (Stamelou & Höglinger, 2016); and selective reuptake inhibitors of serotonin (citalopram), and noradrenaline (atomoxetine) for the treatment of behavioural symptoms in frontotemporal lobar degeneration syndrome, Parkinson's disease and Huntington's disease (reviewed in Rittman et al. (2016), Holland et al. (2021), and Shannon and Fraint (2015). The efficacy of the above agents are however limited by the number of intact synapses, and therefore their utility is restricted to use in mild to moderate disease.

Another approach is to promote synaptic compensation by encouraging the brain's natural ability to compensate for synapse loss in response to neurological diseases or insult. This may include increasing the size of remaining synapses (Scheff, 2003), upregulating synaptic proteins in mild disease (Mukaetova-Ladinska et al., 2000), changes in synapse

dynamics (J. S. Jackson et al., 2017), or changes in synaptic connectivity (Abuhassan et al., 2014). Key to these processes are the growth factors BDNF (brain derived growth factor), NGF (nerve growth factor), and VGF (non-acronymic; nerve growth factor regulated molecule), which are decreased or dysregulated in patients with AD (Cocco et al., 2010; I. Ferrer et al., 1999; Mufson et al., 1995), and HD (reviewed in Tyebji and Hannan (2017)). Intracerebroventricular infusion of NGF, in a phase 1 clinical trial of three AD patients, resulted in some cognitive improvement, albeit outweighed by the side effects relating to the method of delivery (Mandel, 2010). Further evidence on the efficacy of targeting the above neurotrophic factors therapeutically comes from animal studies. For example, in an amyloid precursor protein mouse model of AD, BDNF administration resulted in significant recovery of synaptic loss (Nagahara et al., 2009). Similar evidence is present in mouse models of HD (reviewed in Alberch et al. (2004)), however a recent randomised phase 2 clinical trial of cysteamine (which upregulates BDNF, and also acts through antioxidant mechanisms) failed to show a statistically significant improvement in motor scores in HD patients (Verny et al., 2017)– albeit synaptic density was not measured as an end-point in this trial.

Additional to modulating neurotrophic mechanisms, pathways promoting synaptic recovery have also proven promising. One such pathway includes the wnt pathway – a key modulator of synaptic integrity. Targeting this pathway for example, by blocking the effects of ROCK (Rho-associated kinase – a major cytoskeleton regulator) (Sellers et al., 2018) or DKK-1 (Dickkopt wnt signalling pathway inhibitor 1 – a negative regulator of the wnt signalling pathway) (Marzo et al., 2016) has illustrated positive results in restoring synapses in animal models.

There are currently two clinical trials with synaptic density (or surrogates thereof) as a trial endpoint. The LUCIDITY trial (NCT03446001) is a phase III trial in patients with mild to moderate Alzheimer's disease, employing FDG-PET and cognitive endpoints to assess the effects of a methylene blue derivative (known to disrupt tau-tau binding and therefore stop tau aggregation) on synaptic function, with results expected in mid-2022. UCB-J PET is being used in a second trial to measure the effects of the sigma2 receptor antagonist CT1812 on synaptic density in mild to moderate AD patients (NCT03493282) – results are expected later this year.

A third trial (NCT02167256) also used FDG-PET and cognitive endpoints, to assess the effects of the Src/Abl tyrosine kinase inhibitor saracatinib on synaptic function. Saracatinib (AZ0530) blocks the toxic effects of the fyn kinase pathway which affects tau hyperphosphorylation, as well as promotes beta amyloid toxicity (Kaufman et al., 2015; Nygaard et al., 2014). This drug had offered therapeutic promise in mouse models of AD (APP/PS1), where it led to increased synaptic density in the hippocampus (as measured with UCB-J PET (Toyonaga et al., 2019)) and rescued spatial memory deficits (Kaufman et al., 2015). The trial was however ended in 2019 due to lack of clinical benefit in human participants with AD (van Dyck et al., 2019). In the same year however, a phase 1 study of saracatinib in patients with Parkinson's disease commenced using functional MRI, as well as FDG-PET and electrophysiological measures of brain activity through EEG monitoring – the trial is still ongoing with results expected soon. Clinical trials targeting the neuroinflammatory system in neurodegeneration (for example by using non-steroidal anti-inflammatory drugs, minocycline, or CHF5074 - a microglial modulator, in AD) have illustrated mixed clinical benefit, albeit none directly measure synaptic density as an endpoint (reviewed in Kwon and Koh (2020)). Anti-inflammatory drugs used for the treatment of multiple sclerosis are currently being trialled in patients with motor neuron disease (NCT02469896), with results expected soon.

Therapeutic approaches targeting synapses in human, have been limited thus far, due to the limitations posed by translating animal research into human clinical trials. For example, we have yet to discover a sensitive enough biomarker to measure changes in synapses either over time or in response to therapy. However, advances in the development of UCB-J PET are promising, as well as the developments in detecting fluid biomarkers of synaptic function (for example, CSF neurogranin (Kester et al., 2015), and SNAP25 levels (Brinkmalm et al., 2014)). Moreover, the discovery, and in-human validation of PET ligands measuring mitochondrial complex I ([^{18}F]BCPP-EF PET), and sigma-1R ([^{11}C]SA-4503), as markers of neuronal stress are also promising (Mansur et al., 2020). A second limitation is the difficulty in mimicking human disease in animal models. A possible way of overcoming this limitation is the use of inducible pluripotent stem cells (iPSCs) from patients with neurodegenerative disorders, enabling the study of human disease but also neurodevelopment and neurophysiology in early disease (Taoufik et al., 2018).

Although clinical trials in primary tauopathies have not directly targeted synapses thus far, the lessons learnt from those in other neurodegenerative disorders will be directly applicable given common pathogenic pathways of synaptic toxicity. Current trials in PSP are mainly focused on the removal of, or reductions in, the toxic tau species, in turn reducing tau-induced-synaptotoxity. Potential therapeutic approaches to achieve this aim include: (1) reducing MAPT gene expression through the use of anti-sense oligonucleotide (similar to approaches used in spinal muscular atrophy (Finkel et al., 2017) and Huntington's disease (Pharmaceuticals, 2018)); (2) targeting the post-translational modification pathways - for example, reducing tau hyperphosphorylation, and acetylation which may allow increased clearance through the ubiquitin pathway; (3) preventing tau aggregation; (4) immune neutralisation or clearance of different tau species via either active or passive strategies, thereby preventing cell-to-cell transmission and synaptotoxicity; (5) microtubule stabilisation. Thus far there are 24 tau-targeting therapeutics agents that have gone through, at least, a phase I clinical trial in tauopathies, with a further 15 in development.

Cell-to-cell transmission of pathological tau has been proposed as a key pathogenic method of spread in tauopathies. One therapeutic approach is therefore to prevent this process and the ensuing tau seeding and synapto-toxicity, by using immune-mediated mechanisms (reviewed in VandeVrede et al. (2020) and Giunta et al. (2021)). Monoclonal antibodies against certain pathogenic epitopes of extracellular tau have been trialled in AD, and a few in PSP/CBS with the most recent, using the agent BIIB092 (PASSPORT (NCT03068468), TauBasket study (NCT03658135)), and C2N (NCT02985879), terminating early due to a lack of efficacy on primary or secondary endpoints.

Lastly, given dendritic and synaptic function also occur as a result of loss of cytoskeletal integrity, stabilising the microtubule has been proposed as a potential therapeutic mechanism. However, microtubule stabilising agents davunetide (trialled in PSP) (Boxer et al., 2014) and abeotaxane (a taxane derivative; trialled in PSP and CBS) (Tsai et al., 2020), ended early due to lack of clinical efficacy, and worsening of cognitive symptoms, respectively.

The success of any of the clinical trials summarised above are dependent on a few factors: first the ability of the proposed agents crossing the blood-brain barrier; second, is the

pathological diversity of tauopathies imposing a challenge in finding a common treatment that can be applied to all diseases in which tau plays a toxic role; and third is the limited knowledge about the exact pathogenic region of extracellular tau to target with immune-mediated therapies. An added limitation is the lack of a sensitive enough trial endpoint able to detect clinical efficacy. The PSP rating scale has been used in almost all PSP/CBS trials as the gold standard endpoint and in all has failed to show any change in response to treatment. Whether the latter observation is as a result of lack of clinical efficacy of the agents under investigation, or whether the PSP-rating scale is not a sensitive endpoint is not clear as yet, and warrants further attention (M. K. Chen et al., 2021; Grötsch et al., 2021).

The pathogenic mechanisms leading to cognitive decline in PSP and CBD are complex with synaptic density point of convergence of multiple processes. However, although targeting pathways that lead to synaptic maintenance is promising, we must consider the role environmental, genetic, and epigenetic factors play in the pathogenesis of tauopathies (Litvan et al., 2021). Likewise, we must consider the consequences of clinical benefit in any of the trials above – the success of any agent able to slow down or reverse the pathology in PSP/CBD must be complementary to effective symptomatic management of what will become a chronic condition.

6.5 Overall Study Limitations and future directions

There are limitations to my work. In each of the experimental chapters I discuss key limitations, however, here I reprise the discussion of principal limitations.

Firstly, I used the *clinical diagnostic criteria* for PSP-Richardson's syndrome and amyloid negative CBS (here called CBD) to select a clinical cohort with likely a 4-repeat tauopathy as the underlying pathological diagnosis. Whilst both PSP-Richardson's syndrome and amyloid negative corticobosal syndrome are highly correlated with a 4-repeat tauopathy at post mortem both from our local data and internationally (Alexander et al., 2014; Gazzina et al., 2019; Respondek et al., 2017), other pathologies are possible, and indeed so are coexistent pathologies that may synergistically contribute to neurodegeneration (Robinson et al., 2018). The results in all experimental chapters would be strengthened by neuropathological correlates, to test the correlations between phenotype and pathology, and

between *in vivo* to post mortem measures of synaptic density, as well as tau-to-synapse correlations. Post mortem validation will be available in the next 3-4 years for many pf my participants through the ongoing SENDeR research study. However, given the demanding nature of the protocol, the initial cohort was biased towards patients in earlier stages of disease with a longer interval expected to pathology.

Second, there are limitations posed by potential non-specific binding of particular PET ligands, and by the interpretability of PET signals in relation to their molecular target. For example, in the case of [^{11}C]UCB-J PET the signal has been attributed to presynaptic density. The ligand specifically binds the presynaptic protein SV2A; in this regard the PET signal is a marker of SV2A or synaptic vesicle density which may not necessarily reflect synaptic density at large. However, the number of vesicles per nerve terminal is a stable feature of neurons, as well as the number of SV2A molecules per vesicle – the synaptic pool size is rapidly replenished during neuronal activity to sustain capacity for ongoing neurotransmitter release (Bajjalieh et al., 1993a; Südhof, 2004). Therefore, radioligand binding to SV2A provides an estimate of the density of nerve terminals and serves as a proxy for the quantification of synaptic density (Finnema et al., 2016). No direct measure of postsynaptic density exists currently but specific postsynaptic receptor ligands (for example glutamate or AMPA receptors) are in development.

There are however, increasing debates about the interpretability of the signal from [^{18}F]AV-1451 PET. This ligand was originally developed to bind paired helical filaments (3-repeat/4-repeat tau) of Alzheimer's disease, with very strong *in vivo* and post mortem correlation. Although this radioligand has also been successfully used in non-Alzheimer's pathology, it has a lower affinity for the 4-repeat tauopathy of PSP and CBD, with reports of off-target binding to other non-tau targets (for example monoamine oxidase in the striatum, and neuromelanin in the brainstem) therefore bringing the interpretability of its signal under question (Marquié et al., 2017; Soleimani-Meigooni et al., 2020). Despite the caveats to using this ligand in PSP and CBD, [^{18}F]AV-1451 binding is highest in disease-related subcortical regions of PSP compared to healthy controls (Passamonti et al., 2017; Jennifer L Whitwell et al., 2017b) suggesting that, despite off-target binding, it is able to capture pathological changes compared to controls. In chapter 5, I illustrated that in brain regions beyond those identified as potential sites of off-target binding, there is a strong correlation with synaptic density moderated by disease severity. The [^{18}F]AV-1451 signal

from cortical areas is unlikely to be affected by currently identified off-target binding sites, but further research in this field is required as to the driving pathological factor underlying the PET signal if not tau. The field of *in vivo* tau imaging is still relying on [^{18}F]AV-1451 but next generation radioligands are soon to be utilised as research tools with a higher affinity for 4-repeat tauopathy (on post mortem tissue) and less off-target binding (for example [^{18}F]PI-2620) (Brendel et al., 2020; Kroth et al., 2019; Mormino et al., 2020; A. Mueller et al., 2020). It would therefore be important to replicate the findings in chapter 5 using a next generation tau ligands (e.g. [^{18}F]PI-2620) to further assess the tau/synapse interaction *in vivo.*

The cross-sectional design of my experiments is another limitation. One is unable to draw any conclusion about the progressive change in synaptic density over time, or causation of synaptic loss secondary to tauopathy. There are plans in the SNEDeR study to follow-up patients longitudinally, but this this has been stalled by the Covid-19 pandemic. It is however, reassuring to know that [^{11}C]UCB-J is sensitive to changes in synaptic density over time in another disease context (Glorie et al., 2020), allowing the study of causality, and mediation.

Lastly, I use a relatively small sample size; despite the large effect sizes depicted in chapters 3-5, my cohort may not be representative of the entire PSP population, in that it only included patients with PSP-Richardson's syndrome; and my study was not inclusive of a broad ethnic background. Caution must therefore be taken in generalising the interpretation of the results. Related to this, the study aims to extend PSP recruitment to non-Richardson's syndrome subtypes, and also to early stage disease through the recruitment of asymptomatic MAPT carriers.

6.6 Conclusion

In this thesis, I illustrate the importance of synaptic density, and the consequences of synaptic dysfunction in progressive supranuclear palsy and amyloid negative corticobasal degeneration, as disease models for neurodegeneration. I provide the first *in vivo* evidence for pre- and postsynaptic synaptic change in patients with PSP and CBD and their correlations with disease severity, using a novel PET radioligand [^{11}C]UCB-J. I show that synaptic loss colocalises with other markers of pathology in PSP and CBD. Together, my

results inform the understanding of primary tauopathies, based on *in vivo* assessment in human patients, with direct implications for design of future trials targeting synaptic maintenance and recovery in neurodegeneration.

7 APPENDICES

A. Supplementary materials for Chapter 1

All of A-C must be present for all diagnoses
A. Sporadic occurrence B. Age greater than 40 at first symptom C. Gradual progression of symptoms

Possible PSP-RS
Both A+B must be present A. Slow velocity of vertical saccades B. More than two steps backwards on the pull test within 3 years of symptom onset

Probable PSP-RS
One of A-B must be present A. Vertical supranuclear gaze palsy B. Slow velocity of vertical saccades
One of C-D must be present C. Frequent unprovoked falls within 3 years D. Tendency to fall on pull test within 3 years

Probable PSP-F
One of A-B must be present
A. Vertical supranuclear gaze palsy
B. Slow velocity of vertical saccades
Three of C-G must be present C. Apathy D. Bradyphrenia E. Dysexecutive syndrome F. Reduced verbal fluency G. Impulsivity, disinhibition or perseveration

Possible PSP-CBS
One of A-B must be present A. Vertical supranuclear gaze palsy B. Slow velocity of vertical saccades
One of C-E must be present (asymmetric or symmetric) C. Orobuccal or limb apraxia D. Cortical sensory loss E. Alien limb phenomena
One of F-H must be present (asymmetric or symmetric) F. Limb rigidity G. Limb akinesia H. Limb myoclonus

Possible PSP-SL
One of A-B must be present A. Vertical supranuclear gaze palsy B. Slow velocity of vertical saccades
One of C-D must be present C. Meets criteria for non-fluent variant primary progressive aphasia D. Progressive apraxia of speech

Definite PSP
Neuropathological diagnosis with any clinical presentation

Exclusion criteria
There are 10 absolute and 25 relative exclusion criteria. See Höglinger et al. 2017 for details Important exclusion criteria include: A. Prominent episodic memory impairment suggestive of Alzheimer's Disease B. Prominent autonomic failure suggestive of multiple system atrophy of Lewy body disease C. Prominent, unexplained visual hallucinations or fluctuations in alertness D. Sudden onset or stepwise progression of symptoms suggestive of vascular aetiology

Figure A1. Progressive Supranuclear Palsy Diagnostic Criteria.
A selection of the 8 subtypes of PSP are outlined in this figure; please refer to Hoglinger 2017 for full details of all subtypes. It is not unusual for patients to meet criteria for several PSP subtypes, in which case the Multiple Allocations eXtintion (MAX) rules criteria (Grimm et al. 2019) suggest that a diagnosis of PSP-Richardson's Syndrome and/or higher levels of diagnostic certainty are prioritised.

Probable CBS
Both A+B must be present A. Asymmetric presentation of two or more of A1-A3 A1. Limb rigidity or akinesia A2. Limb dystonia A3. Limb myoclonus B. Two or more of B1-3 B1. Limb or orobuccal apraxia B2. Cortical sensory deficit B3. Alien limb phenomena (more than simple levitation)

Possible CBS
Both A+B must be present A. Asymmetric or symmetric presentation of one or more of A1-A3 A1. Limb rigidity or akinesia A2. Limb dystonia A3. Limb myoclonus

Definite Corticobasal degeneration (CBD)
Neuropathological diagnosis

Figure A2. Corticobsal Syndrome Diagnostic Criteria (Armstrong *et al*. 2013a).

B. Supplementary materials for Chapter 4

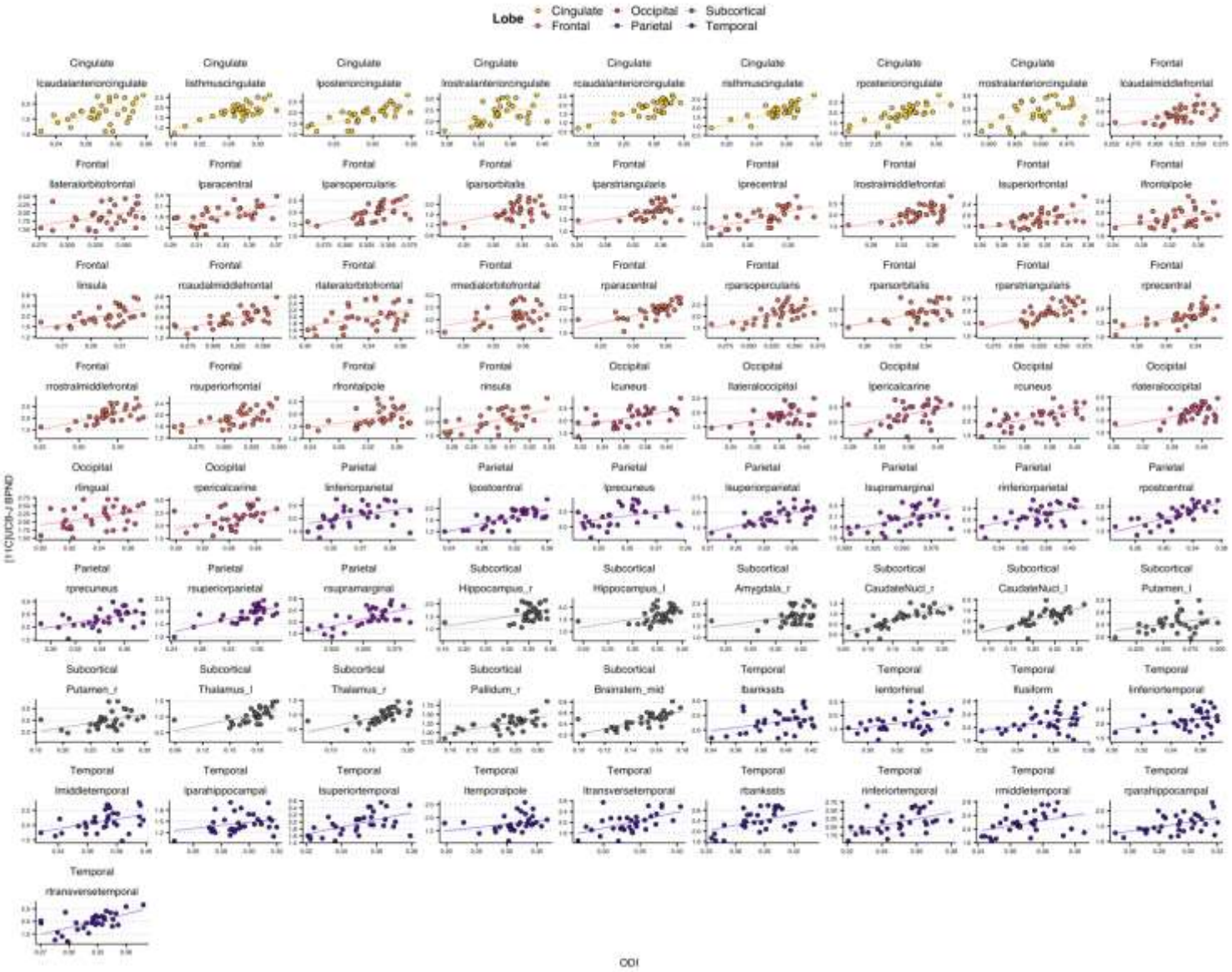

Figure B1. Regional association between cortical ODI and [^{11}C]UCB-J BP_{ND} in PSP-RS and CBD.
Significant regional associations are observed within the patient group (FDR $p < 0.05$, after adjusting for age and local GM atrophy).
Abbreviation: ODI = Orientation Dispersion Index; FDR = False Discovery Rate; GM = Grey Matter.

C. Supplementary materials for Chapter 5

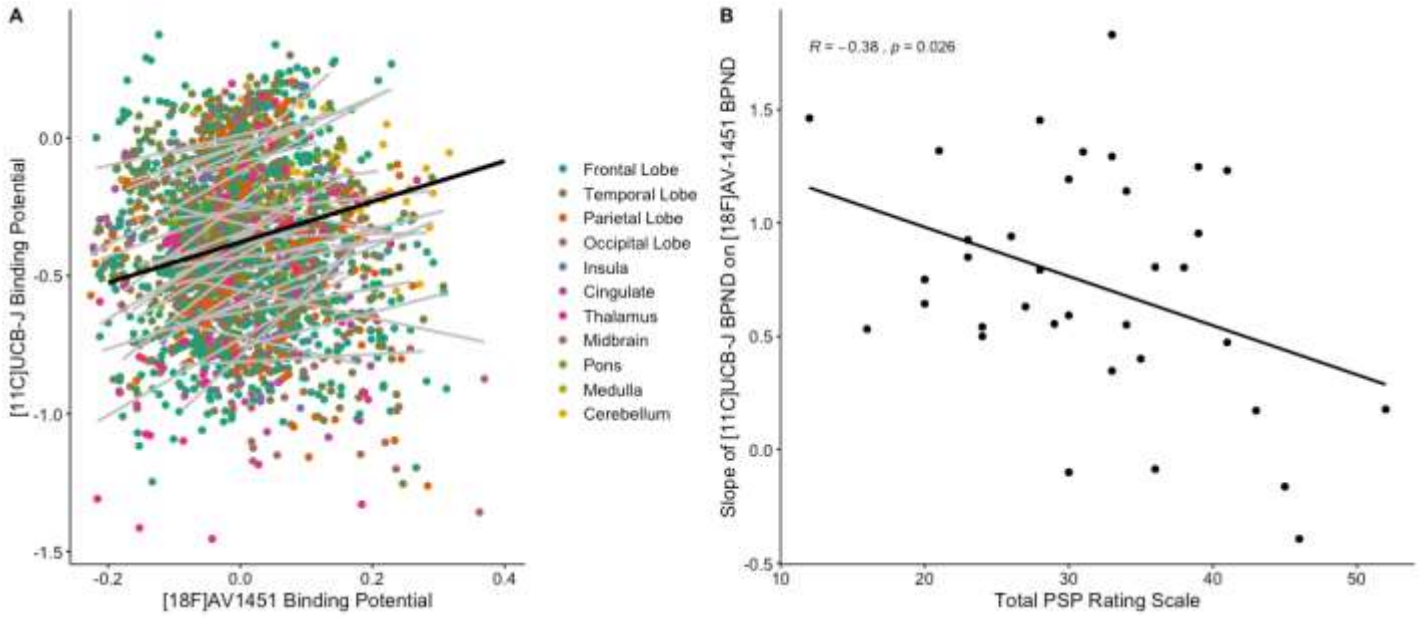

Figure C1. The association between synaptic density ([^{11}C]UCB-J) and molecular pathology ([^{18}F]AV-1451) is a function of disease severity.
A) Scatter plot [^{11}C]UCB-J and [^{18}F]AV-145 partial volume uncorrected BP_{ND} from 23 patients with PSP and 12 patients with amyloid-negative corticobasal syndrome . Each grey line in A represents a patient's data across 73 regions of interest (excluding those with previously reported off-target binding, i.e. basal ganglia and substantia nigra); the black line illustrates the overall model fit from equation 2. B) The slope for each individual (i.e. each grey line in A) is negatively correlated with disease severity (as measured with the PSP rating scale); R= -0.38, p = 0.03.

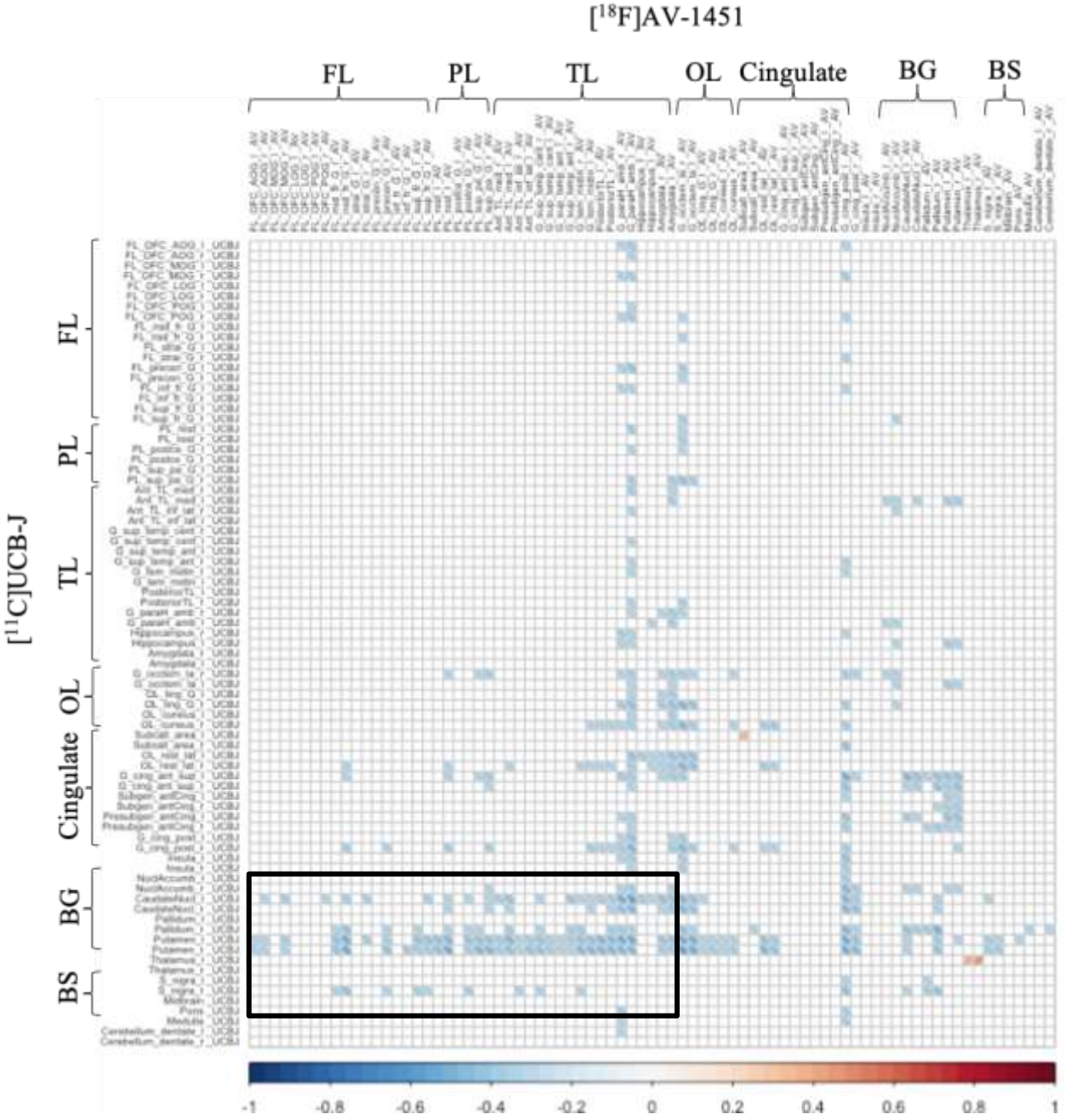

Figure C2. Cortical molecular pathology is negatively correlated with subcortical synaptic density (Partial Volume Uncorrected). Correlation between [^{18}F]AV-1451 BP_{ND} in a source region (horizontal axis) and [^{11}C]UCB-J BP_{ND} in a target region (vertical axis), across 79 regions of interest in patients, using partial volume uncorrected binding potentials. The black box in the lower left quadrant focuses on cortical [^{18}F]AV-1451 BP_{ND} and subcortical [^{11}C]UCB-J BP_{ND}. Abbreviation: l: left, r:right, FL: Frontal Lobe, OFC: Orbitofrontal Cortex, AOG: Anterior Orbital Gyrus, MOG: Middle Orbital Gyrus, LOG: Lateral Orbital Gyrus, POG: Posterior Orbital Gyrus, mid_fr_G: Middle Frontal Gyrus, strai_G: Straight Gyrus, precen_G: Precentral Gyrus, inf_fr_G: Inferior Frontal Gyrus, sup_fr_G: Superior Frontal Gyrus; PL: Parietal Lobe, postce_G: Postcentral Gyrus, sup_pa_G: Superior Parietal Gyrus: TL: Temporal Lobe, Ant_med: Anterior Medial, Ant_inf_lat: Anterior Inferiolateral, "G_sup_temp_cent: Superior Temporal Gyrus – superior part, G_sup_temp_ant: Superior Temporal Gyrus- Anterior part, G_tem_midin_r: Middle and Inferior Temporal Gyrus, G_paraH_amb: Parahippocampal and ambient gyri, G_occtem_la: Occipitotemporal Gyrus – Lateral Part (Fusiform Gyrus), OL_ling_G_l: Lingual Gyrus, Subcall_area: Subcallosal Area, OL_rest_lat: Latera Remainder of Occipital Lobe, G_cing_ant_sup: Cingulate Gyrus Anterior Part, Subgen_antCing: Subgenual Frontal Lobe, Presubgen_antCing: Presubgenual Frontal Lobe, G_cing_post: Cingulate Gyrus – posterior part; Fl: Frontal Lobe; TL: Temporal Lobe; PL: Parietal Lobe; OL: Occipital Lobe; BG: Basal Ganglia; BS: Brainstem. Uncorrected for multiple comparison.

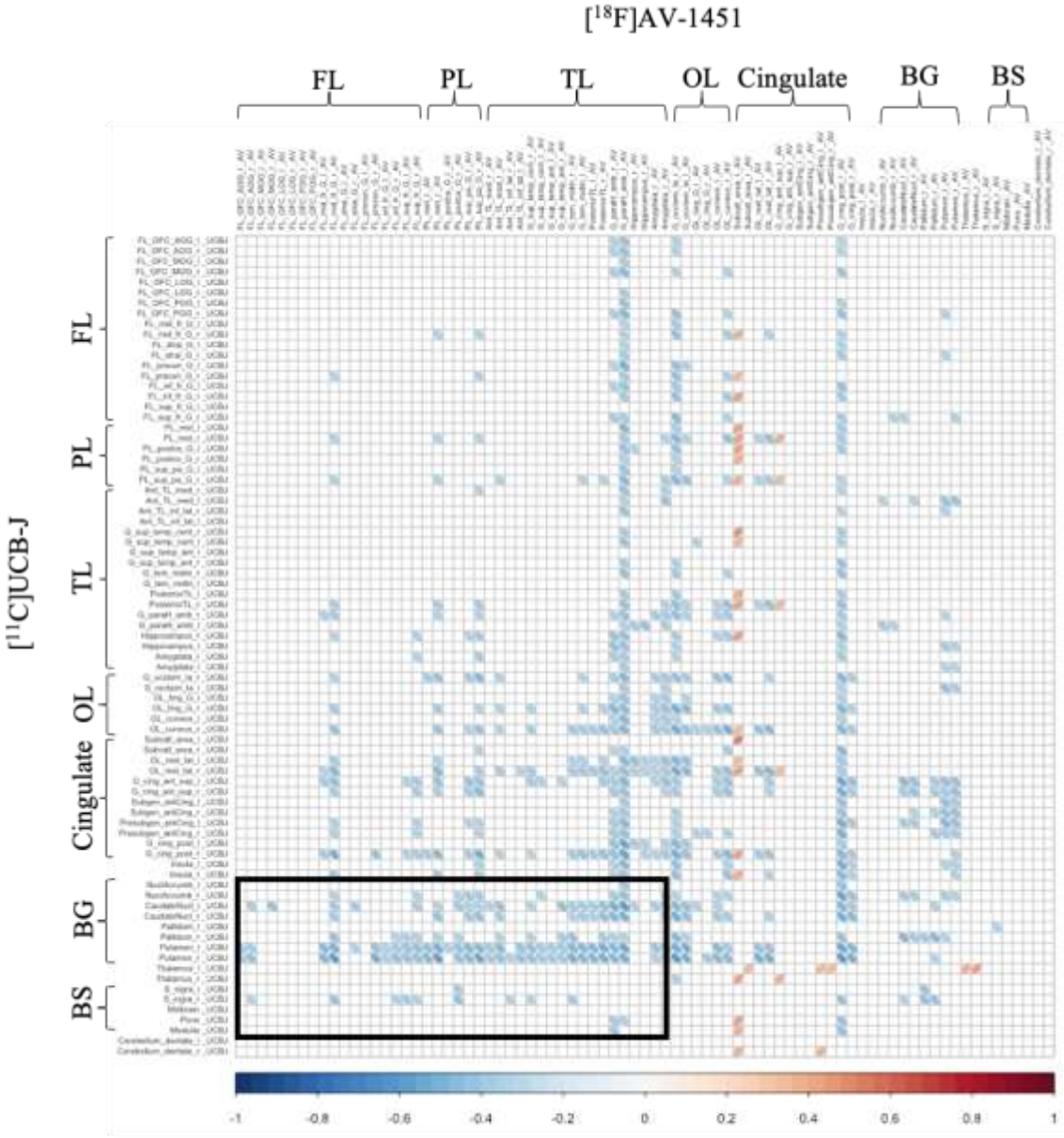

Figure C3. Cortical molecular pathology is negatively correlated with subcortical synaptic density (Partial Volume corrected).
Correlation between [^{18}F]AV-1451 BP_{ND} in a source region (horizontal axis) and [^{11}C]UCB-J BP_{ND} in a target region (vertical axis), across 79 regions of interest in patients, using GTM partial volume corrected binding potentials. The black box in the lower left quadrant focuses on cortical [^{18}F]AV-1451 BP_{ND} and subcortical [^{11}C]UCB-J BP_{ND}.. Abbreviation: l: left, r:right, FL: Frontal Lobe, OFC: Orbitofrontal Cortex, AOG: Anterior Orbital Gyrus, MOG: Middle Orbital Gyrus, LOG: Lateral Orbital Gyrus, POG: Posterior Orbital Gyrus, mid_fr_G: Middle Frontal Gyrus, strai_G: Straight Gyrus, precen_G: Precentral Gyrus, inf_fr_G: Inferior Frontal Gyrus, sup_fr_G: Superior Frontal Gyrus; PL: Parietal Lobe, postce_G: Postcentral Gyrus, sup_pa_G: Superior Parietal Gyrus: TL: Temporal Lobe, Ant_med: Anterior Medial, Ant_inf_lat: Anterior Inferiolateral, "G_sup_temp_cent: Superior Temporal Gyrus – superior part, G_sup_temp_ant: Superior Temporal Gyrus- Anterior part, G_tem_midin_r: Middle and Inferior Temporal Gyrus, G_paraH_amb: Parahippocampal and ambient gyri, G_occtem_la: Occipitotemporal Gyrus – Lateral Part (Fusiform Gyrus), OL_ling_G_l: Lingual Gyrus, Subcall_area: Subcallosal Area, OL_rest_lat: Latera Remainder of Occipital Lobe, G_cing_ant_sup: Cingulate Gyrus Anterior Part, Subgen_antCing: Subgenual Frontal Lobe, Presubgen_antCing: Presubgenual Frontal Lobe, G_cing_post: Cingulate Gyrus – posterior part; Fl: Frontal Lobe; TL: Temporal Lobe; PL: Parietal Lobe; OL: Occipital Lobe; BG: Basal Ganglia; BS: Brainstem. Uncorrected for multiple comparison.

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