

Positron Emission Tomography Imaging of the Cellular Phase of Alzheimer's Disease

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Abstract

Alzheimer's Disease (AD) is the commonest fatal neurodegenerative disease causing dementia. It has a devastating effect on individuals and their families and causes progressive cognitive, social and functional decline with no current cure. AD is characterised neuropathologically by amyloid- β ($A\beta$) and tau protein deposition, but this does not fully explain how the brain is affected and why neurodegeneration occurs. Key molecular drivers of disease could include cell stress and impaired mitochondrial oxidative phosphorylation with synaptic loss. Elucidating key mechanisms of this disease process *in vivo* has the potential for better diagnostic classification, and a better understanding of the neurobiology in order to prioritise targets for earliest interventions to delay or reverse disease.

In this study I set out to determine whether multimodal imaging using PET (amyloid-beta, the sigma-1 receptor, mitochondrial complex I, synaptic vesicle 2A) complemented by MR (structural, arterial spin labelling and neurite orientation diffusion density imaging) can determine key clinically relevant cellular mechanisms in AD patients.

Chapter 1 provides an introduction to AD and imaging. Chapter 2 outlines the material and methods used in this study.

In Chapter 3 I aimed to boost the diagnostic power of one key trigger of AD - amyloid- β , using [^{18}F]Florbetapir PET, with machine learning applied to big data. I created an algorithm that utilised spatial information by k-means clustering then an optimised quadratic support vector machine based on the progression of regional brain vulnerabilities to AD across 758 volunteers from the Alzheimer's Disease Neuroimaging Initiative (ADNI). I was able to develop an automated diagnostic decision support and show that my algorithm outperforms classifiers proposed previously for amyloid- β PET.

In Chapter 4 I aimed to understand the underlying mechanisms of AD using multiple imaging modalities in my highly phenotyped cohort of early AD and controls. I quantified the *in vivo* density of the endoplasmic reticulum cell stress marker, the sigma 1 receptor (S1R) using [^{11}C]SA4503 PET, as well as that of mitochondrial complex I (MC1) with [^{18}F]BCPP-EF and the pre-synaptic vesicular protein SV2A with [^{11}C]UCB-J. I also integrated these assessments with regional brain volumes and brain perfusion (CBF) measured with MRI arterial spin labelling and followed the AD patients longitudinally to estimate rates of change with disease progression over 12-18 months. I generated new evidence for widespread cellular stress and bioenergetic abnormalities in early AD and showed how they may be clinically meaningful.

In Chapter 5 I sought to understand synaptic microstructure from my early AD cohort in further detail. I quantified *in vivo* measures of microarchitecture

evaluating extracellular free water (FISO), neurite density (NDI) and orientation dispersion (ODI) using neurite orientation dispersion imaging (NODDI), as well as more conventional DTI measures of fractional anisotropy (FA), mean/axial/radial diffusivity (MD, AD, RD, respectively), and the pre-synaptic vesicular protein SV2A with [^{11}C]UCB-J PET in my early AD cohort compared to controls. My results showed increased extracellular free water (FISO) using NODDI MRI could be more sensitive to differences in pathology related to synapse loss that could be used to support early-stage evaluations of novel therapeutics for AD

Overall, my study provides a systematic investigation of the cellular phase of AD using PET and MR imaging. My main finding was that widespread cell stress in AD leads to regionally vulnerable expression of pathology. If these findings were applied to both clinical practice and drug discovery they could lead to better diagnostic classification and prioritise targets to modulate the disease.

Contents

	Page
Abstract	3
Declaration of Originality	7
Publications	9
Grants & Prizes	15
List of Figures	16
List of Tables	20
Acknowledgements	23
Abbreviations	26
Chapter 1: Introduction	30
Chapter 2: Materials and Methods	63
Chapter 3: Boosting the diagnostic power of amyloid- β PET using a data driven spatially-informed classifier for decision support	85
Chapter 4: Widespread cell stress and mitochondrial dysfunction in early Alzheimer's Disease	110
Chapter 5: Synaptic microstructure <i>in vivo</i> in early Alzheimer's Disease	149
Chapter 6: Summary and Future Directions	175
Chapter 7: References	183

Declaration of Originality

The data presented in this thesis is my own original work and has been appropriately referenced. Specifically I have done the following:

Study 1: I conducted all aspects of this study using the ADNI dataset from conceptualization, design and methodology, coding and software development, formal analysis, data curation, visualization, to writing of the original draft, reviewing and editing through to publication.

Study 2 and Study 3: I was involved in the early stages of conceptualization and methodology of the larger MINDMAPS study. I wrote the initial protocol and conducted all aspects of this study from study regulation (REC, ARSAC, Sponsor Approval – all documents/meetings), wrote clinical progress reports, conducted daily project administration, wrote all clinical manuals/instructions, lay summary documents and held patient focus groups. I conducted all of the investigation (recruitment, logistics, informed consent, screening, physical health checks, neuropsychological tests, bloods, cannulation, radioligand injection, PET and MR scanning), as well as carrying out all formal analysis, data curation, and visualization. I wrote the original draft and revised and edited this for publication.

Please see the section on acknowledgements for all contributions to this work.

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Grants and Prizes

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2019	Guarantors of Brain Travel Grant £1000
2019	Imperial College Department of Medicine Travel Award £650
2019	Brain and Brain PET Early Career Investigator Travel Bursary £1078
2018	Alzheimer's Society Clinical Research Fellowship, £159,658 (PI)

List of Figures

Figure 1.1: Illustrative proportional aetiological differences of young onset dementia compared to late onset dementia.

Figure 1.2: The pathogenesis of Alzheimer's Disease.

Figure 1.3: An overview of PET imaging.

Figure 1.4: Tracer uptake and compartmental models

Figure 1.5: Diagram illustrating PET molecular targets in this study used to image the cellular phase of AD.

Figure 2.1: Overview of study methods to boost the diagnostic power of A β PET

Figure 3.1: GM masked images showing the mean SUVR, standard deviation SUVR, and Z-score images stratified by clinical group

Figure 3.2: K means voxel-wise clustering results mapped to anatomy.

Figure 3.3: Mean number of voxels of each group for A β clusters 1-4 and A β clusters 1-4 map overlaid on MNI 152.

Figure 3.4: Overlays of composite region of interest, GM, and A β clusters 1-4 on MNI152.

Figure 3.5: Violin plots showing the distribution of number of positive amyloid voxels in each individual for A β clusters 1-4 stratified by clinical group.

Figure 3.6: AUC-ROC curve diagram of classification of AD vs CN for A β clusters 1-4 compared to and combined with other methods

Figure S3.1: Number of voxels of each group within each k division.

Figure S3.2: Silhouette plots proximity of cluster to voxels in neighbouring clusters.

Figure S3.3: WM SUVr between clinical groups.

Figure 4.1: Cross-sectional and longitudinal results for volume and CBF

Figure 4.2: Mean voxel-wise PET images for controls, AD, and z-score contrasting AD from controls.

Figure 4.3: Cross-sectional differences between AD patients and controls, and longitudinal differences for AD patients between S1R, MC1, and SV2A.

Figure 4.4: Associations between MC1 and SV2A in the hippocampus for controls and AD patients.

Fig.4.5: Voxel-wise Z score PET images of longitudinal change in AD patients.

Figure S4.1: Association between hippocampal [^{18}F]BCPP-EF DVR_{CS} and visuospatial scores

Figure S4.2: Associations between hippocampal [^{11}C]UCB-J DVR_{CS} and language scores

Figure S4.3: Longitudinal associations with frontal lobe [^{18}F]BCPP-EF DVR_{CS} and [^{11}C]SA4503 V_T/f_p with ACE memory

Figure S4.4: Pearson's r correlation matrix showing correlations between regional mean $[^{11}\text{C}]\text{SA4503 } V_T/f_P$, $[^{18}\text{F}]\text{BCPP-EF } \text{DVR}_{\text{CS}}$, $[^{11}\text{C}]\text{UCB-J } \text{DVR}_{\text{CS}}$, volume, ASL and clinical variables at baseline and longitudinally for AD.

Figure S4.5: MC1, SV2A and S1R voxels with a hyperplane fitted to the data for CN, a CN hyperplane fitted to AD, and these mapped to anatomy

Figure S4.6: Figure caudate MRI fits in AD

Figure 5.1: Cross-sectional percentage differences for individual AD patient relative to the control mean for NDI, ODI, FISO, SV2A and normalised brain volume measures.

Figure 5.2: AD z score images on MNI152 for $[^{11}\text{C}]\text{UCB-J } \text{DVR}_{\text{CS}}$ PET (SV2A) and NODDI images of free water (FISO) neurite density (NDI) and orientation dispersion (ODI). Spatially normalised AD patient images acquired at the study baseline were contrasted to those from the control group. Thresholded voxel-wise z-scores $((\text{AD patients mean} - \text{controls mean})/(\text{controls standard deviation}))$ are illustrated, using the group mean control image as a template.

Figure 5.3: Significant negative associations between the normalised hippocampal volume and regional FISO in AD patients.

Figure 5.4: A negative association between SV2A and FISO (A) and a trend to a negative association for SV2A and RD (B) in the parietal lobe in AD were found.

Figure 5.5: Loss of SV2A (A) and higher FISO (B) both were associated with impaired language (picture naming and semantic fluency) in the parietal lobe of AD patients.

Supplementary Figure S5.1: DTI cross-sectional results for FA, MD, L1, RD with box and whisker plots of individual AD patient percentage differences from the mean controls corresponding with values in Supplementary Table 2.

Supplementary Figure S5.2: Significant positive associations between regional FISO and RD in AD patients for the parietal lobe (A) and hippocampus (B) were found.

Supplementary Figure S5.3: Pearson's r correlation matrices describe correlations between regional mean neurite density (NDI), orientation dispersion (ODI), free water (FISO), SV2A [^{11}C]UCB-J DVR_{CS}) with partial volume correction, normalised brain volume and clinical variables for controls (n=16) (A) and AD (n=12) patients (B).

List of Tables

Table 1.1: Specific domains assessed in a full work up of a patient with cognitive impairment.

Table 1.2: List of some common, potentially reversible, and rare/untreatable causes of young onset dementia.

Table 2.1: Total potential maximum doses in mSv received by participants.

Table 3.1: Clinical demographics of participants

Table 3.2: Median, interquartile range (IQR) and skewness of A β positive voxels in clusters 1-4 across diagnostic category.

Table 3.3: Classification results comparing sensitivity, specificity, accuracy, AUC-ROC and error rates for different methods.

Table 3.4: Percentage change compared to the proposed method - 6 features (A β clusters 1-4 and GM voxels and GM SUVR))

Table 4.1: Cross-sectional demographic and neuropsychological testing data for AD patients and cognitively normal controls at baseline

Table 4.2: Neuropsychological test results for AD patients after 12-18 months

Table S4.1: Baseline [^{11}C]SA4503 V_T/f_p (sigma 1 receptor), [^{18}F]BCPP-EF DVR_{CS} (mitochondrial complex I), [^{11}C]UCB-J DVR_{CS} (synaptic density), MRI ASL cerebral blood flow (CBF) (all partial volume corrected) and normalised brain volumes (volume) measures in AD patients (n=12) and controls (n=16).

Table S4.2: Mean [^{11}C]SA4503 V_T/f_P (sigma 1 receptor), [^{18}F]BCPP-EF DVR_{CS} (mitochondrial complex I), [^{11}C]UCB-J DVR_{CS} (SV2A synaptic density), MRI ASL cerebral blood flow (CBF) (all partial volume corrected) and normalised brain volumes (volume) measures in AD patients after 12-18 month follow up.

Table S4.3: Mean baseline [^{11}C]SA4503 V_T/f_P (sigma 1 receptor), [^{18}F]BCPP-EF DVR_{CS} (mitochondrial complex I), [^{11}C]UCB-J DVR_{CS} (synaptic density) (*without* partial volume correction) and normalised brain volumes (volume) measures in Alzheimer's patients (n=12) and controls (n=16).

Table S4.4: Mean [^{11}C]SA4503 V_T/f_P (sigma 1 receptor), [^{18}F]BCPP-EF DVR_{CS} (mitochondrial complex I), [^{11}C]UCB-J DVR_{CS} (synaptic density), MRI ASL cerebral blood flow (CBF) (*without* partial volume correction) and normalised brain volumes (volume) measures in Alzheimer's patients after 12-18 month follow up

Table 5.1: Cross-sectional demographic and neuropsychological testing data for AD patients and controls at baseline.

Table 5.2: Cross-sectional effect sizes using Hedges g between ROIs for SV2A PET, volume, NODDI FISO, NDI, ODI, and DTI FA, MD, L1 and RD for AD patients compared to controls.

Supplementary Table S5.1: Baseline neurite density (NDI), orientation dispersion (ODI), free water (FISO), [^{11}C]UCB-J DVR_{CS} (synaptic density) with partial volume correction, and brain volume measures in AD patients and controls.

Supplementary Table S5.2: Baseline DTI measures for FA, MD, L1, RD in AD patients and controls.

Supplementary Table S5.3: Neurite density (NDI), orientation dispersion (ODI), free water (FISO), [^{11}C]UCB-J DVR_{CS} (synaptic density) with partial volume correction, and brain volume measures in AD patients after 12-18 month follow-up.

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Dedication

To Roohi, Lara, Kian, Mum and Dad

Abbreviations

[¹¹C] 11-Carbon

[¹⁸F] 18-Fluorine

1TC One-Tissue Compartment

2TC Two-Tissue Compartment

ACE III Addenbrooke's Cognitive Examination III

AD Alzheimer's Disease

AD Axial Diffusivity

ADNI Alzheimer's Disease Neuroimaging Initiative

APP Amyloid precursor protein

ASL Arterial spin labelling

ATP Adenosine Triphosphate

AUC Area under curve

Aβ Amyloid-β

BBB Blood Brain Barrier

BCPP-EF 18F-2-tert -butyl-4-chloro-5-{6-[2-(2- fluoroethoxy)-ethoxy]-pyridin-3-ylmethoxy}-2H-pyridazin- 3-one

BP_{ND} Binding potential non displaceable

C9orf72 Chromosome 9 open reading frame 72

Ca²⁺ Calcium

CBF Cerebral Blood Flow

CDR Clinical Dementia Rating

CN Cognitively Normal

CNS Central nervous system

CS Centrum semiovale

CSF Cerebrospinal fluid

CT Computerised Tomography

DLPFC Dorso lateral pre frontal cortex

DTI Diffusion tensor imaging

DVR Distribution volume ratio

EMCI Early mild cognitive impairment

ER Endoplasmic Reticulum

ETC Electron Transport Chain

FA Fractional anisotropy

FISO Fraction of isotropic

fP Plasma free fraction

FTD Frontotemporal dementia

GM Grey matter

IRE1 Inositol-requiring enzyme 1 α

KeV kiloelectronvolts

Ki Inhibitor constant

LMCI Late mild cognitive impairment

MA1 Multilinear Analysis 1

MAM Mitochondria-Associated Membrane

MAPT Microtubule associated protein tau

mAs Milliampere seconds

MBq Megabecquerel

MC1 Mitochondrial Complex I

MCI Mild cognitive impairment

MD Mean diffusivity

mDa Megadalton

MMSE Mini mental state examination

MNI Montreal Neurological Institute

MRI Magnetic Resonance Imaging

ms Millisecond

mSv Millisievert

NART National Adult Reading Test

NDI Neurite Density Index

NIA-AA National Institute on Aging and Alzheimer's Association

nM Nanomolar

NODDI Neurite Orientation Density and Dispersion Imaging

Nrf2 nuclear factor erythroid 2-related factor 2

ODI Orientation dispersion index

OXPHOS Oxidative Phosphorylation

PET Positron Emission Tomography

PRGN Progranulin

PSEN Presenilin

PVC Partial Volume Correction

PVE Partial Volume Effects

RBANS Repeatable Battery for Assessment of Neuropsychological Status

RD Radial Diffusivity

RF Radio Frequency

ROI Region of Interest

ROS Reactive Oxygen Species

S1R Sigma 1 Receptor

SA4503 (1-(3,4-dimethoxyphenethyl)-4-(3-phenylpropyl)piperazine dihydrochloride)

SPECT Single photon emission computerized tomography

SPM12 Statistical Parametric Mapping version 12

SRTM Simplified Reference Tissue Model

SUVr Standardised Uptake Value ratio

SV2A Synaptic Vesicle Protein 2A

TAC Time Activity Curve

UCB-J ((R)-1-((3-((11)C-methyl-(11)C)pyridin-4-yl)methyl)-4-(3,4,5-trifluorophenyl)pyrrolidin-2-one)

UPR Unfolded Protein Response

V_T Volume of distribution

WM White matter

Chapter 1

Introduction

Abstract: Alzheimer's Disease (AD) is the commonest fatal neurodegenerative disease causing dementia with no current cure. The onset of disease follows brain cellular homeostatic collapse over a long prodromal period. Since the first described case of AD, clinical diagnosis and stratification has been challenging and involves a detailed history of the chronology of a variety of neuropsychiatric symptoms. Diagnostic frameworks in research have subsequently evolved over many years shifting gradually from the clinical syndrome to the neurobiology. AD is characterised neuropathologically by amyloid- β ($A\beta$) and tau protein deposition, but these do not fully explain how the brain is affected and why neurodegeneration occurs. Molecular drivers of AD include cell stress and impaired mitochondrial oxidative phosphorylation and neuroinflammation with cortical microstructural changes and synaptic loss. Advances in brain imaging in AD, particularly PET and MR imaging, hold great promise in the investigation of the underlying neurobiology and underpinning these are analytical and modelling challenges. Specific cellular PET radioligands targeting $A\beta$, endoplasmic reticulum cell stress (S1R), mitochondrial oxidative phosphorylation (MC1) and synaptic density (SV2A), alongside structural MR, cerebral perfusion and neurite orientate diffusion density imaging and advances in machine learning have only recently allowed *in vivo* characterisation of the disease. Together these enable investigation of the cellular phase of AD using PET and MR imaging as described in chapters 2-5.

Alois Alzheimer in 1907 first described a case of “An Unusual Illness of the Cerebral Cortex” describing the mental state and psychopathology of the 51 year old Auguste Deter at the Asylum for the Insane and Epileptic in Frankfurt. (1) He described neuropsychiatric symptoms of memory loss, disorientation in time, place and person, pathological jealousy, and paranoid delusions with auditory hallucinations. His subsequent cognitive testing showing immediate memory deficits, dysgraphia, severe aphasia and notable inability to describe common objects such as using the words “milk-pourer” instead of cup. His neurological examination revealed normal eye movements, reactive pupils and normal patella reflexes. He described a progressive deterioration in symptoms over four and half years until she became bed bound, incontinent, developed bedsores and required complete nursing care.

Following Auguste’s death Alzheimer described his findings at post-mortem showing an evenly atrophic brain without macroscopic focal degeneration, the vascular tissues as having arteriosclerotic change, fibrils of unique thickness, and glia with numerous fibers. In his conclusions he urged the clinician not to be satisfied in classifying underdetermined cases by forcing them into existing categories of recognized illness and suggested that the only way to make progress was to study both clinical and pathological assessments together.

Today, Alzheimer’s Disease (AD) is known as a fatal brain disease causing progressive cognitive, social and functional decline. AD is the largest cause of

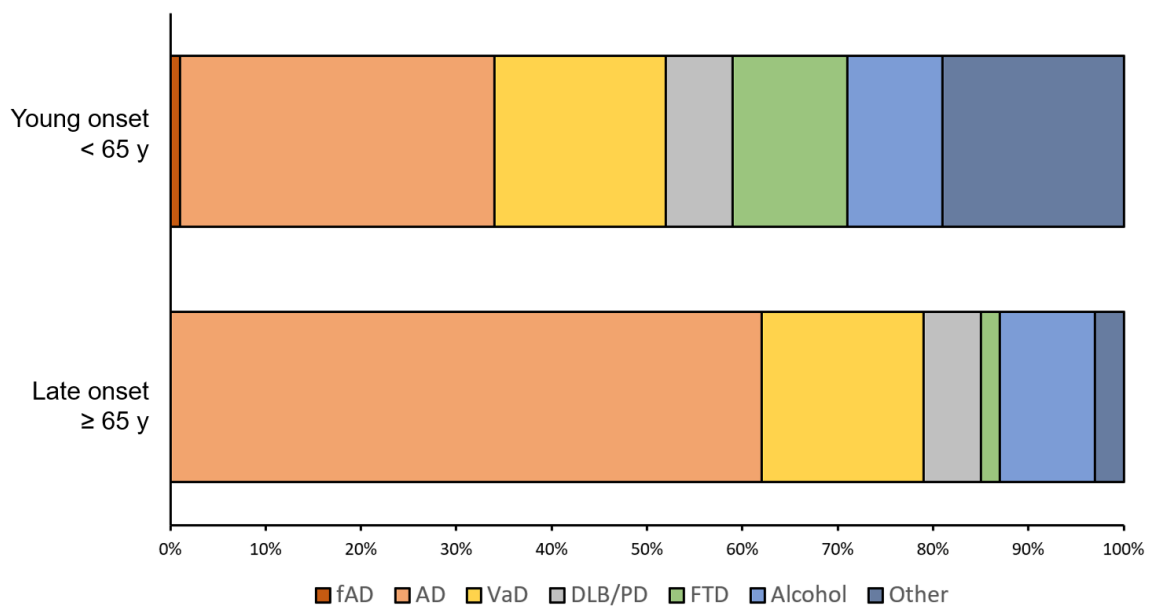
dementia and affects 50 million people worldwide. It has a major impact on individuals, their families and clinical care as well as larger society. (2) Current dementia costs to the global economy are \$818 billion/year. (3) It is forecast that the absolute number of people with AD will triple by 2050, (4,5) and 20% of women and 10% men will develop AD. (6,7) At present, there is no current cure with the best available symptomatic treatments unable to change disease course and prognosis.

Challenges of diagnosis

Despite awareness of AD and an increased realisation of the burden of disease, the nuances of diagnosis and decreased awareness of the signs and symptoms make accurate diagnosis and discrimination from other illnesses challenging.

The most common presentation of late onset AD is in patients over the age of 65 with multiple co-morbidities. Insidious onset symptoms typically involve episodic memory impairment and an amnesic presentation before progressing to multiple domain dysfunction with accompanying behavioural and psychological symptoms. In patients younger than 65 this is still generally still true, but ruling out other causes/mimics of dementia becomes more important. (8)

Figure 1.1 Illustrative proportional aetiological differences of young onset dementia compared to late onset dementia. Data combined from (9–11). Data on mixed pathology not available for both groups. fAD = familial autosomal dominant Alzheimer’s, AD = Alzheimer’s disease, VaD = Vascular dementia, DLB/PD = dementia with Lewy bodies/Parkinson’s disease, FTD = frontotemporal dementia.



The disease commonly follows a heterogenous course following a long asymptomatic prodromal period. Clinically, most information is gained from a detailed history of the nature and crucially the chronology of an individual’s symptom to form an impression of disease trajectory spanning the Alzheimer’s continuum. (12) This requires a careful collateral history, most frequently from a spouse or other family member. Specific domains are assessed in addition to a

general medical, psychiatric and medication history and full neurological examination.

Table 1.1 Specific domains assessed in a full work up of a patient with cognitive impairment. (8)

Memory	Disinhibition/Sexual behaviour
Language	Appetite/Food preference
Numerical Skills	Motivation/Apathy
Executive skills	Anxiety/Agitation
Visuospatial skills	Delusions/Hallucinations
Neglect phenomena	Activities of daily living
Visual perception	Occupational history/performance
Route finding/landmark identification	Family history of dementia
Mood	Alcohol and drugs
Personality and social conduct	Risk to themselves/others

In an ideal environment this is followed by specific investigations to rule in/rule other causes, especially potentially reversible ones (see Table 1.2). This is commonly considered used more in younger patients.

Table 1.2 List of some common, potentially reversible, and rare/untreatable causes of young onset dementia.

Common	Potentially Reversible	Rare
Alzheimer's disease	Depression	Progressive
Vascular dementia	Alcohol related brain damage	supranuclear palsy
Frontotemporal dementia	Endocrine (hypothyroidism, Cushing's, Addison's)	Multiple system atrophy, Corticobasal degeneration
Dementia with Lewy bodies	B1/B12/B6 deficiency	Huntington's disease
	Benign tumours	Creutzfeldt–Jakob disease
	Normal pressure hydrocephalus	Progressive multifocal leucoencephalopathy
	Infections – HIV/syphilis, Whipple's disease	Subacute sclerosing panencephalitis
	Limbic encephalitis (paraneoplastic/autoimmune)	Leucodystrophies.
	Inflammatory (vasculitis, multiple sclerosis, sarcoid)	Neuronal ceroid lipofuscinosis (Kuf's disease).
		Cerebral autosomal dominant arteriopathy with subcortical infarcts and leucoencephalopathy (CADASIL).
		Mitochondrial encephalopathies

Mental state examination including assessing appearance/behaviour, speech (rate/tone/volume), mood, thought form/content, perceptions (hallucinations/illusions), cognition, and insight into the nature and degree of the illness, capacity and risk to themselves/others is necessary both to diagnose and decide appropriate management.

Cognitive testing such as the Mini-mental state examination (MMSE) and Addenbrookes Cognitive Assessment (ACEIII) is valuable both to assess baseline but also for future comparison to evaluate disease trajectory and response to treatment. Further detailed neuropsychology may also prove useful.

Bloods to exclude reversible and other causes (FBC, U&E, CRP, LFTs, B12/Folate, TFTs, Glucose) and consideration of infectious causes e.g. delirium, and HIV/syphilis), alongside consideration of focussed genotyping for ApoE and in younger onset patients for the underlying cause (PSEN/APP in Alzheimer's and C9orf72, MAPT, PRGN for suspected FTD may be indicated, particularly in cases of diagnostic ambiguity and a number of neurogenetics centres offer dementia gene panels including these and other relatively frequently found mutations.

Brain imaging – namely structural MR (T1/T2) ideally would be carried out in suspected dementia patients to rule in and rule out diagnoses unless there are specific contraindications. Generally, even with expert neuroradiology input, MRI scans can appear normal in the early stages of neurodegenerative disease. FDG-

PET, Amyloid PET and SPECT are also considered in the diagnostic workup (13–15) in order to distinguish types of neurodegeneration. CSF for beta-amyloid 1-42 and tau may be considered alongside identification of inflammatory causes and chronic infections. (16)

Following diagnostic work up there are often lots of mixed and uncertain pictures. The Alzheimer's syndrome generally involves initial memory deficits secondary to dysfunction of medial temporal lobe structures, specifically the entorhinal cortex and hippocampus. As the disease progresses, patients develop impairment in executive function, attention, language, visuospatial function, personality and behaviour, and eventually motor control. This is mirrored by increasing dysfunction/atrophy in parietal and frontal regions, leading eventually to disability and increasing dependence. There is also increasing neuropsychiatric involvement including apathy, depression, delusions, hallucinations and agitation. (8)

Diagnostic criteria

Given the variety of neuropsychiatric signs and symptoms and heterogenous course of the disease it has become increasingly important to further classify AD and the only way to do so is to understand more about the underlying biology. An international diagnostic framework of AD only emerged decades following Alzheimer's first case report. In 1984 initially the core clinical concept of AD by the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) and the Alzheimer's Disease and Related Disorders Association

(ADRDA) was formed. (17) The concept of probable AD during life based on clinical features and only definitive diagnosis from biopsy or autopsy emerged.

By 2007 biomarkers of AD were added to the framework, and the ABCDE criteria were formed, citing the need to diagnose earlier in the disease course. (18) Probable AD consisted of the presence of an early episodic memory impairment with supportive features of medial temporal lobe atrophy, abnormal CSF amyloid or tau, specific [^{18}F]FDG PET bilateral temporo-parietal hypometabolism, or proven autosomal dominant AD mutation.

In 2014 this clinical biological AD type was simplified requiring the presence of either a typical or atypical phenotype, and a biomarker consistent with AD pathology such as abnormal amyloid/tau ratio on CSF, positive amyloid PET, or an autosomal dominant mutation (PSEN1/2 or APP). MRI and FDG PET was lost from the core diagnostic criteria but suggested to be useful in monitoring. (19)

By 2018 a movement towards a more biological definition of AD gained momentum with the formation of the ATN (amyloid, tau and neurodegeneration) classification system. (20) This shifted the definition from the syndromal to the biological by incorporating an evolving framework where new biomarkers could be added to enable a more precise approach to interventional trials and prodromal AD. This definition resulted in AD referring to A β plaques and pathologic tau deposits, defined *in vivo* by abnormal biomarkers. Alzheimer's pathologic change was in the early stage of Alzheimer's continuum, defined *in vivo* by an abnormal A β biomarker with normal pathologic tau biomarker. The Alzheimer's clinical

syndrome was the recommended terminology for clinically amnesic syndrome or a syndromal variant applying to both patients with both mild impairment and dementia.

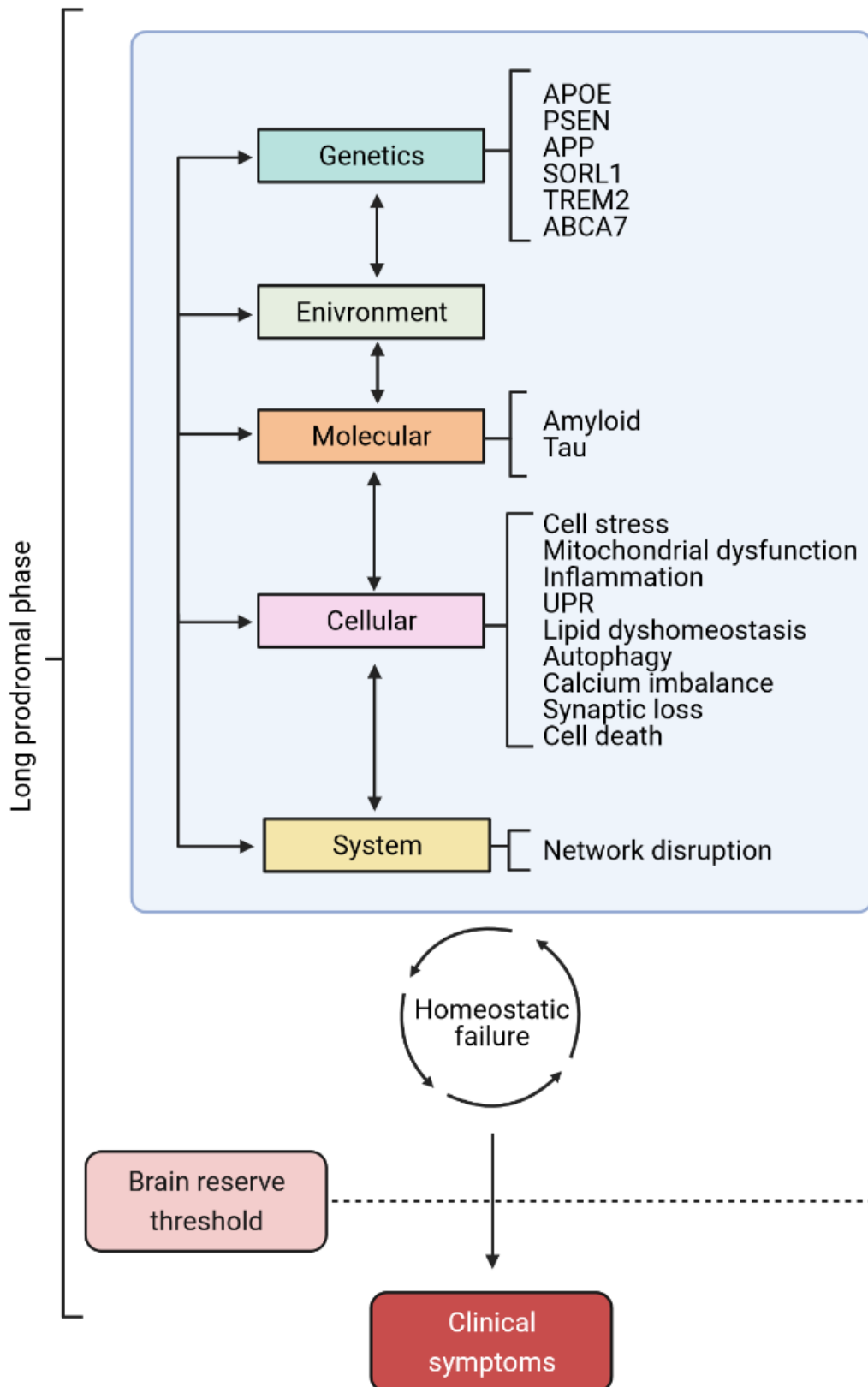
These diagnostic criteria crystalized following increasing research into the underlying neurobiology of AD.

Neurobiology of AD

Understanding the neurobiology of AD has the potential to show greater insight into the disease process. It promises to provide a stronger basis for the development of targeted therapeutics and also provide better dynamic biomarkers of progression.

Late onset AD is polygenic in origin (21) and has a non-linear multi-system model of progression involving many parallel pathways (Figure 1.2). These consist of feedback loops of genetic and environmental influences, neuroinflammation (22), lipid dysregulation, proteostatic impairment of autophagy, proteopathies (23,24), the unfolded protein response and cellular stress driving mitochondrial oxidative phosphorylation inhibition (25,26), calcium dysregulation, bioenergetic deficits, cerebrovascular insufficiency (27) and neuronal loss (28) as outlined below.

Figure 1.2 The pathogenesis of Alzheimer's Disease. AD has a long prodromal phase with multiple pathophysiological mechanisms from genes and environment, to molecular, cellular and system level homeostatic failure with multiple feedback loops that when surpassing a particular threshold result in clinical symptoms.



Classical features of early AD are the relatively focal nature of major pathological structural and functional changes,(29–32) despite common expression of genetic determinants of susceptibility throughout the brain. This focal appearance has been interpreted as a reflection of a selective vulnerability of those regions, determined by factors such as their connectivities, functional activities or metabolism. (33,34) Although not implicated in genetic susceptibility to AD and therefore unlikely to be causal, mitochondrial dysfunction and reduced oxidative phosphorylation have long been recognised in AD. (35,36) Amyloid- β toxicity and impaired autophagy/mitophagy are amongst the mechanisms hypothesised to contribute to this.

Neuropathologically, AD has a dynamic protein deposition phase involving amyloid- β (A β) (23,37,38) and neurofibrillary tau tangle deposition, (29) in addition to neuropil threads, dystrophic neurites, reactive astrogliosis and microglial cell activation. (39) Specific lesions including Hirano bodies and granulovacuolar degeneration in the hippocampal formation and loss of synaptic elements form core features in AD brains. (40,41) A pattern of deposition of amyloid and tau (in various pathogenic forms) has emerged based on post mortem brains and validated in vivo using PET. (32,42,43) Characteristically amyloid is detected through the disease stages initially neocortically, then allocortically, at the diencephalic nuclei, striatum then cerebellum. (30) Tau is found in translimbic

areas, limbic areas, and then in the entorhinal cortex then isocortex as the disease progresses (29,44).

Synaptic loss, which has a mechanistically proximate relationship to neuronal circuit dysfunction and cognitive symptoms, is an early secondary pathology related both to amyloid- β toxicity, tau as well as to inflammatory glial activation. (45) Neuronal loss at post mortem has been shown to largely parallel the latter process in particular. Loss of absolute synaptic numbers in AD has been shown to be associated with cognitive dysfunction. (40,46) The induction of early synapse damage with retrograde axonal degeneration and dendritic tree atrophy has also been shown. (39,41,47) Amongst these specific changes are abhorrent neurite sprouting and reduced synaptic exosome proteins finally culminating in vesicular machinery vulnerability and neuronal circuit dysfunction. (48–50) Inexorable neurodegeneration progresses and is associated with tau deposition, (32,51) and seen as regional atrophy globally, and focally in the medial temporal lobes and hippocampus (31,52) as seen on MR imaging.

Together these are believed to culminate in disease presentation 15-25 years after initiation of pathology following homeostatic collapse after a long prodromal cellular phase. (50) The role of increased oxidative stress and an unfolded protein response (UPR) have been highlighted by biochemical and transcriptomic data, as well as genetic and biochemical evidence for early lipid dyshomeostasis. (53)

Multiple factors contribute to cell stress in AD. Variants in genes encoding proteins involved in lipid processing, phagocytosis and inflammation are associated with a genetic risk for AD. These proteins are ubiquitous throughout the brain, and biochemical and transcriptomic investigations suggest that dysfunction or dysregulation of these pathways all contribute to cell stress. (54) The sigma 1 receptor (S1R) is upregulated in the endoplasmic reticulum (ER) at sites of cell stress caused by impaired mitochondrial oxidative phosphorylation. (55,56) Oxidative stress is a major pathogenic mechanism in AD (57), although ER stress (e.g., from protein misfolding) may also contribute. (58)

Cell stress due to oxidative damage and the UPR may disrupt brain homeostatic mechanisms across multiple cell types by assessing the sigma 1 receptor (S1R). S1Rs are pluripotent modulators of multiple systems (59) and expressed widely through the CNS (60) with no similarity to any other human protein (61). The S1R is primarily at the ER-mitochondrion interface (MAM), but is also present on the nucleus membrane, and can relocate to plasma membrane in the presence of agonists. (62) S1R are believed to promote cellular survival by ensuring Ca^{2+} signalling from the ER into mitochondria (via IP3), enhancing ER–nucleus signalling to induce antioxidant release (via IRE1), and attenuating free radical damage (via Nrf2). (55,59) In AD ER-mitochondrion crosstalk occurring via S1R–IP3R–VDAC linkages have a role in disease effects and losing MAM integrity by S1R knockdown are speculated in development of AD. Preclinical models show S1R knockdown causes degeneration of mouse hippocampal neurons (25) with

disturbance of ER-mitochondria S1R connections resulting in the unfolded protein response and activation helping with cell survival. (56)

Brain metabolism is disrupted as the disease progresses (35,63–67). Failure of MAM regulation of calcium signalling, which is essential for ATP generation, may contribute to neuronal loss and disease presentation. (68,69) Mitochondrial complex I, the largest complex in the electron transport chain (~1 MDa), is responsible for the respiratory activity and oxidative phosphorylation (OxPHOS) in mitochondria (70) and crucial for ATP energy production, and in turn essential for life. Mitochondria also have critical cellular roles in calcium homeostasis, generation of ROS and cellular signalling. (71,72)

In addition to cell stress and OxPHOS, loss of synaptic density (28) has been associated with the amnestic presentation of the disease. Changes in synaptic density are likely earlier than neurodegeneration with changes previously seen in brain tissue at *post mortem* or biopsy. (46,73) Recently these changes have been able to be visualised directly *in vivo* in humans (74) with PET ligands targeting the synaptic vesicle glycoprotein 2A (SV2A) forming a useful investigative tool to measure synapses. (75) SV2 is a 12 transmembrane domain integral protein with three isoforms (2A, 2B and 2C). SV2A is the most ubiquitous and is expressed as a transmembrane glycoprotein in secretory vesicles on presynaptic terminals. It is critical to synaptic function, particularly Ca^{2+} dependent exocytosis. (76,77)

Furthermore, vascular insufficiency and blood-brain barrier disruption has been shown to occur in early AD (78–80) with causative associations shown on capillaries, arterioles and arteries, and more recently, pericytes following amyloid. (81)

The long prodromal phase of AD pathology suggests that the healthy brain has considerable functional reserve. (82) Functional reserve can be defined operationally as the extent to which redundancy or plasticity allows normal cognition to be maintained despite an impairment of functions (e.g., metabolic, synaptic or neuronal circuit) necessary to support it. Metabolic reserve can be defined as the level to which a metabolic process (such as oxidative phosphorylation) can be reduced before becoming rate limiting, e.g. for performance on standard cognitive tests. Synaptic reserve can be defined similarly in terms of the loss in synapse density in functionally relevant brain regions that can occur without associated impairment of related cognitive performance. Identification of pathological processes affecting pathways without sufficient functional reserve supports prioritisation of those pathways as therapeutic targets for AD, as the correlation of function and cognitive performance suggests that their modulation could lead to measurable clinical benefits.

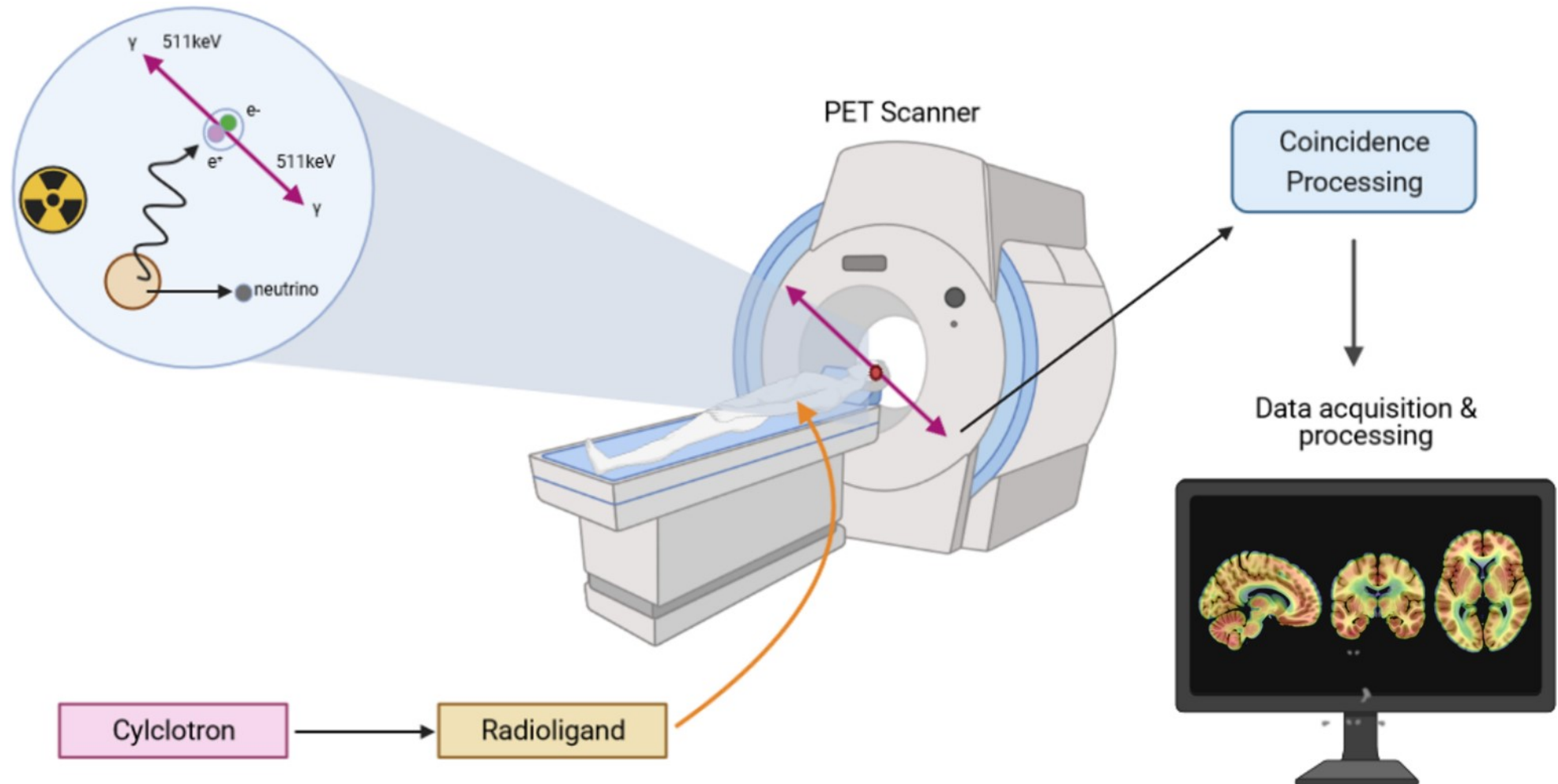
PET imaging

Molecular imaging using PET is the only way of visualising targets of interest *in vivo* in AD patients. It is based on the principle of injecting a microdose of a

radioligand (radiolabelled molecule) that crosses the blood brain barrier and binds to the target of interest in the brain.

Radioligands are produced by a cyclotron and tagged usually with [^{11}C] or [^{18}F]. [^{11}C] has a short half-life of ~ 20 minutes, [^{18}F] ~ 120 minutes. After injection, positron (β^+) decay leads to annihilation of an electron and emits two perpendicular 511keV photons or gamma rays. These are detected by gamma detectors (scintillator crystals and photomultiplier tubes) located in the scanner around the individual's brain when they arrive at the detector simultaneously. Coincidence processing allows spatial reconstruction of the distribution of the radiolabelled molecule. Absolute concentrations measurements of the radioligand over time combined with tracer kinetics models allow precise estimation of the amount retained in a brain tissue of interest and the delivery rates to tissues. PET imaging data is then structurally co-registered with MRI scan data to allow precise anatomical localisation of the signal. (83,84)

Figure 1.3 An overview of PET imaging showing that a specific radioligand is produced by a cyclotron, and following injection into the patient, radioactive decay occurs where a positron annihilates an electron and two perpendicular gamma rays are emitted that are detected by coincidence events and corrected and processed to view the radioactivity in a given ROI.



The scanner resolution depends on the detector size, positron range and photon-non linearity. The assumption is that gamma rays are perpendicular but in practice this varies slightly. Measurement errors that therefore need correction include errors in detectors, and both scattered and random coincidence detection that are not reflective of true coincidence events. Correction is also required as photons in the centre of the body have greater attenuation than more peripherally. Correction of attenuation is performed with a CT scan which allows an attenuation factor calculation for each voxel to be applied to the raw PET data. (83)

Any given voxel will have spill-in signal from adjacent voxels and spill-out signal to adjacent voxels. These are known as partial volume effects (PVE) which may require correction. The finite spatial resolution of the scanner and the voxel size at which the image is sampled are causes of PVE. This is particularly important in AD given widespread cortical and medial temporal atrophy and ventricular expansion. (85) The degree of PVE is influenced by the original structure size with greater effects seen in areas with the greatest atrophy. The aim of partial volume correction (PVC) is to account for the PVE and generate a truer representation of the underlying neurobiology in a given area. There are various methods to conduct PVC, each of which has its own assumptions and limitations. The Müller-Gärtner method (86) has been used in this study. In an ideal investigation both corrected and uncorrected data will show a similar direction of change across specified ROIs.

Following correction for the above measurement errors the PET image is reconstructed with motion correction to reflect radioactivity concentration calibrated both in relation to the individual scanner, and relative to patient arterial blood samples. (83) The radioactivity concentration derived from the PET image across a given area over time forms a time activity curve (TAC). An ROI from an atlas applied to the structural MRI is spatially aligned and registered to a subject's PET image and then often transformed into standard common space for comparison across subjects such as MNI 152. A TAC is usually generated from the mean intensity of voxels in a given ROI and plotted over time.

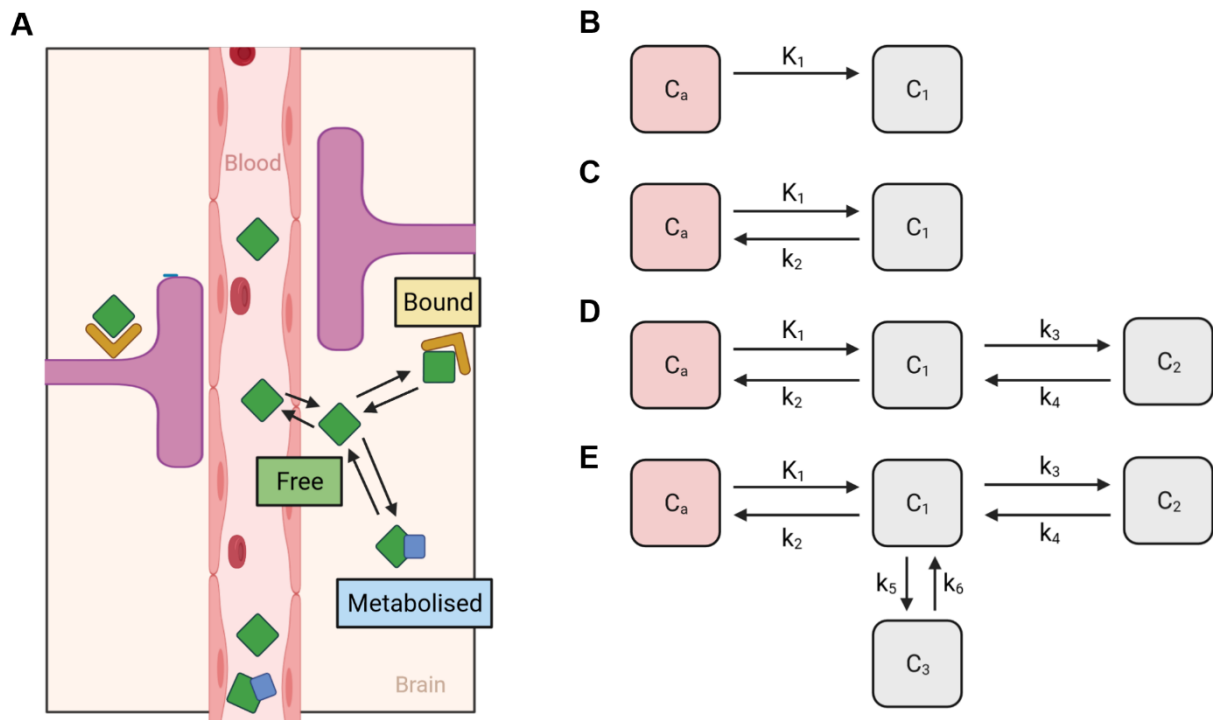
Modelling of dynamic PET data allows quantitative measurements of the proportion of specific and non-specific signal in an area of interest. Kinetic modelling attempts to account for the proportion of radioligand signal in each compartment within a given area. (87) Each compartment refers to the tracer's physical location and its chemical state. One compartment may have many chemical states of a tracer together.

Following injection of a tracer, arterial inflow delivers the tracer to the ROI and venous outflow will carry it away. The tracer will cross the capillary membrane and enter the tissue, where it exists as either free, bound (reversibly or irreversibly) to the target, or metabolised. The free or metabolised tracer exits the tissue. (Figure 1.4 panel A). Compartmental models describe both uptake and clearance of the tracer where C_a is the concentration of tracer in arterial blood, and C_1 , C_2 , C_3 refer

to the concentration of tracers in each compartment, where k_1 to k_6 represent rate constants defining the rate of movement of the radiotracer between each compartments (Figure 1.4, panel B-E).

Another increasingly used model is called graphical analysis which is an alternative method to compartmental modelling. Here radioactivity in arterial circulation and in the tissue can be transformed into linear equations and used to derive physiological meaning. Graphical methods include the Logan plot, (88) and alternative methods to reduce noise such as the multilinear analysis 1 (MA1) model. (89)

Figure 1.4 A shows a radioactive tracer being delivered to brain tissue via arterial inflow. In the tissue it may exist as free, bound to target, or metabolised, and the original or metabolised tracer may return to venous circulation. B shows a one tissue compartment model with irreversible uptake, C shows a one tissue compartment model with reversible uptake, D shows a one tissue compartment model with reversible uptake, D shows a two tissue compartment model and E shows a three tissue compartment model.



After solving a model to best reflect the most accurate tracer concentration in a given voxel, there are various outcome measures that can be used to quantify PET

activity depending on the tracer and its validation methods such as V_T , V_T/f_p , BP_{ND} , $DVR-1$, SUV_r . The V_T is the ratio of the concentration in tissue of radioligand to that in plasma at equilibrium which can also be derived from differential equations of compartmental models. V_T is affected by both plasma protein binding and non-specific binding to non-targets in tissue. To overcome this V_T can either be corrected for the plasma fraction (V_T/f_p) to account for the proportion of radioligand in plasma not bound to its target, or use binding potential, or BP_{ND} which can be derived to remove these confounders. BP_{ND} is the ratio of specifically bound tracer to nondisplaceable tracer in tissue and can be directly calculated from, for example, a reversible 2TC where it is equivalent to the product of the free fraction of ligand in non-displaceable tissue, receptor density and receptor affinity. An equivalent to BP_{ND} , the $DVR-1$ is also a common outcome parameter which is used as a ratio compared to a pseudo-reference region of low binding such as the centrum semiovale where

$$DVR_{CS} = \frac{V_T^{ROI}}{V_T^{CS}} - 1$$

with V_T^{ROI} being the V_T in the region of interest and V_T^{CS} being for example the V_T in the centrum semiovale. A SUV_r outcome measure relies on the existence of a reference region that has low/no specific binding, and is used in place of an arterial input, and describes tissues using the 1TC model. (90)

Magnetic Resonance Imaging

MRI is an imaging technique based on the spin properties of hydrogen nuclei in response to a magnetic field. Hydrogen nuclei within a magnetic field align and precess at a rate governed by the Larmor frequency. Following placement of an individual in the scanner, radiofrequency pulses allow measurements of both longitudinal and transverse magnetic field relaxations, and the varied dynamics of relaxations allow discrimination of the tissue type in a given area. In the case of structural MRI this allows quantification of grey matter, white matter and CSF and when applied to PET imaging signal allows co-registration and spatial localisation. (91)

MRI can be further used to quantify cerebral blood flow using arterial spin labelling (ASL) which magnetically tags hydrogen atoms in blood water which after normalisation allows the generation of a perfusion weighted time activity curve based on the magnetic signal decay. (80) This is then allows measures of absolute perfusion reflecting ml/gram of tissue per minute.

To further characterise microstructural changes using MRI Neurite Orientation Dispersion Imaging (NODDI) can be used. This is an advanced approach to diffusion-weighted MRI based on a multi-compartment diffusion model that allows more precise characterisations of water diffusion microstructure than does diffusion tensor imaging (DTI). (92) When applied to brain grey matter, measures related to the *extracellular* free water (FISO), neurite density (NDI) (the *intracellular* volume

fraction), and the local diffusion tensor orientation dispersion (ODI) (interpreted in this context as a measure of neurite branching).

Imaging amyloid- β

A β is seen as a necessary element as either a cause or response (20) in the development of AD. (93) Amyloid PET *in vivo* correlates well with *post mortem* A β deposition, which was previously the gold standard prior to PET radioligands targeting amyloid. (13) Amyloid PET, originally based on thioflavin-T binding to fibrillar A β (94) has been associated with faster memory decline (95) and regional hypometabolism in connected distal areas. (33) It has emerged as useful for the diagnosis of patients with cognitive decline in clinic (96,97) increasing the accuracy of diagnosis resulting in changing patient management (14,97–99) and as an endpoint measure in clinical trials. (100–102) Commonly available fluorinated radiotracers include [^{18}F]Florbetaben and Florbetapir.

Imaging the sigma 1 receptor

PET ligands targeting the sigma 1 receptor (S1R) form a potentially useful investigative tool to measure activity at the mitochondrial associated membrane, as a marker cell stress. S1R is 25kDa and has a trimeric architecture with a single transmembrane domain in each protomer. The structure has relatively recently been established and it has no similarity to any other human protein. (61) S1Rs are a

pluripotent modulator of multiple systems (59) and expressed widely through the CNS. (60)The S1R is primarily at the ER-mitochondrion interface (MAM), but is also present on the nucleus membrane, and can relocate to plasma membrane in the presence of S1R agonists. (62) S1R are believed to promote cellular survival by ensuring Ca^{2+} signalling from the ER into mitochondria (via IP3), enhancing ER–nucleus signalling to induce antioxidant release (via IRE1), and attenuating free radical damage (via Nrf2). (55,59)

In AD ER-mitochondrion crosstalk occurring via S1R–IP3R–VDAC linkages have a role in disease effects and losing MAM integrity by S1R knockdown are speculated in development of AD. Preclinical models showing knockdown causes degeneration of mouse hippocampal neurons (25) with disturbance of ER-mitochondria S1R connections resulting in the unfolded protein response and activation helping with cell survival. (56)

[^{11}C]SA4503 was developed as a viable PET tracer to image the S1R (103) (104), with optimal imaging methodology in humans recently established (105) in order to assess stress response pathways that are critical for cellular homeostasis in AD. One previous study reported that S1R binding was lower in AD compared with controls (106), but the investigators scanned participants who were being treated with donepezil, which confounds interpretation as it has affinity for the S1R.

Imaging mitochondrial complex I

MC1, the largest complex in the electron transport chain (~1 MDa), is responsible for the respiratory activity and oxidative phosphorylation in mitochondria, and is composed of over 40 subunits. (70) The mitochondrial respiratory chain or electron transport chain (ETC) is crucial for ATP energy production, and in turn essential for life. Neuronal tissue is highly dependent on this energy source and MC1 is known to be the binding site of the inhibitor rotenone. [^{18}F]BCPP-EF was developed as a viable tracer to image MC1 (107–111) with the first full human characterisation recently completed. (105,112)

[^{18}F]BCPP-EF is a pyridazinone analog, has a high affinity for MC1 ($K_i=2.31\text{ nM}$), binding to the PSST subunit. (107,108) The specificity of binding has been shown by competitive inhibition with rotenone in a dose dependent manner *in vitro* in slices (107), *in vivo* in rats (109), and *in vivo* in rhesus monkeys. (111) BCPP compounds have recently been shown to inhibit binding of 3H-dihydrorotenone to MC1 and the proton pumping activity of MC1 in dose-dependent manner *in vitro* in cardiomyocytes. (110) Pre-clinical dosimetry has shown high uptake in the brain and heart *in vivo* in rats. (107) Rhesus monkey [^{18}F]BCPP-EF has shown suitable kinetics for human evaluation. (113)

Studies from non-human primates have shown an age related reduction in monkeys in MC1 activity in all brain regions. (111) A significant negative correlation has only been shown between [^{18}F]BCPP-EF V_T and [^{11}C]PIB SUVR in aged rhesus monkeys. (114) The first full human characterisation of [^{18}F]BCPP-EF and test-

retest reliability was recently completed. (105,112) In AD mitochondrial dysfunction in the parahippocampus has recently been shown with PET in AD patients compared to controls (115) and associated with tau load but used [^{11}C]PBB3 which may be confounded by off-target binding. (116)

Imaging Synapses

PET ligands targeting the synaptic vesicle glycoprotein 2A (SV2A) allow a measure of synapses. (75) SV2 is a 12 transmembrane domain integral protein with three isoforms (2A, 2B and 2C). SV2A is the most ubiquitous and is expressed as a transmembrane glycoprotein in secretory vesicles on presynaptic terminals. It is critical to synaptic function, particularly Ca^{2+} dependent exocytosis (76) and is known to be the binding site of levetiracetam. (77)

[^{11}C]UCB-J was developed as a PET tracer to image SV2A and has shown favourable brain uptake, kinetics, and dosimetry shown in non-human primates. (117) There is a high correlation between *in vitro* SV2A UCB-J binding and *in vitro* synaptophysin density ($r^2 = 0.90$ for GM regions) pre-clinically. (74) [^{11}C]UCB-J has a high affinity for SV2A ($K_i = 7\text{nm}$) and has been successfully displaced by levetiracetam *in vivo* in humans, with good dosimetry (4.5 mSv/MBq). (74) Regional time activity curves showed [^{11}C]UCB-J has fast kinetics, was well described by a 1TC model, or SRTM (reference centrum semi-ovale) and had a 3-9% mean test-retest variability in V_T across regions. (118)

Lower [^{11}C]UCB-J binding was first shown in temporal lobe epilepsy and mesial temporal sclerosis patients (n=3), revealing region specific unilateral decreases in the hippocampus. (74) The first study in MCI/AD using [^{11}C]UCB-J compared A β + patients (n=10) with age-matched A β - controls and showed a significant reduction in hippocampal SV2A binding (41% lower BP_{ND}) that survived partial volume correction and correlated significantly with episodic memory (118) with only modest cortically differences. More recently SV2A PET imaging has been shown in AD and controls (119,120) with widespread reductions in medial temporal and neocortical regions using a cerebellar reference region.

Multiple other candidates selective to SV2A have been developed recently including [^{18}F]UCB-H displaying a good signal but higher variability than [^{11}C]UCB-J (121) and a human dosimetry of 19.7 mSv/MBq. (122)

Aims

The **first aim** of my thesis is to develop and test an algorithm to provide automated decision support in diagnosing AD using a large cohort across the AD continuum from ADNI. This will be based on the progression of regional brain vulnerabilities utilising machine learning methods that are tested for their associations by diagnostic category and disease severity, compared against existing methods..

The **second aim** is to evaluate the underlying neurobiology of AD in a small and highly phenotyped cohort of patients with AD compared with controls, and also longitudinally in patients with AD in order to investigate key mechanisms of cellular pathology. Specifically these will assess cell stress via the sigma 1 receptor (S1R) using [^{11}C]SA4503 PET, oxidative phosphorylation with [^{18}F]BCPP-EF PET, synaptic loss with [^{11}C]UCB-J and measures of regional brain volumes and brain perfusion using structural MR and ASL. Exploratory clinical associations with these measures will also be evaluated.

Building on this, the **third aim**, is to conduct a more detailed study of synaptic cortical microstructure and its associations with synapse loss using MRI neurite orientation dispersion and density imaging (NODDI) and [^{11}C]UCB-J PET in order to find accessible and scalable measures of synapse loss or neuroinflammation associated with synapse loss that are applied to my highly phenotyped cohort.

Hypotheses

1) A β deposition is a necessary cause or response to the cellular pathology of AD but usual interpretation of amyloid PET does not fully utilise all information regarding the spatial distribution of signal. I hypothesise that I am able to develop a data driven, spatially-informed classifier that is able to boost the diagnostic power of amyloid PET in AD using machine learning that will outperform other spatial methods to provide automated decision support for the clinician.

2) Mitochondrial dysfunction leading to significant cellular energy dysregulation is a common feature of neurodegenerative disorders, including AD. Cellular stress is associated with mitochondrial dysfunction and reduction in mitochondrial expression and function, and will lead to in an upregulation of S1R expression in the MAM. Once this compensation fails, synaptic dysfunction and ultimately synaptic loss are a consequence of the deficits in mitochondrial energy production. I expect AD patients will demonstrate higher S1R expression than healthy controls, due to increased cellular stress, and lower expression of MC1 and SV2A, as well as lower brain perfusion and regional volumes compared to healthy controls, and I am able to measure these changes longitudinally.

3) Clinical-pathological correlations suggest a strong relationship between loss of synaptic integrity and cognitive dysfunction. AD pathogenesis proposes that amyloid oligomers induce maladaptive microglial synaptic pruning that leads to

dendritic and axonal degeneration with the loss of neuropil that is believed to be a major contributor to the loss of brain volume as the disease progresses. I expect AD patients will demonstrate higher free water, lower neurite density and orientation dispersion and lower expression of SV2A compared to healthy controls reflecting this.

Chapter 2

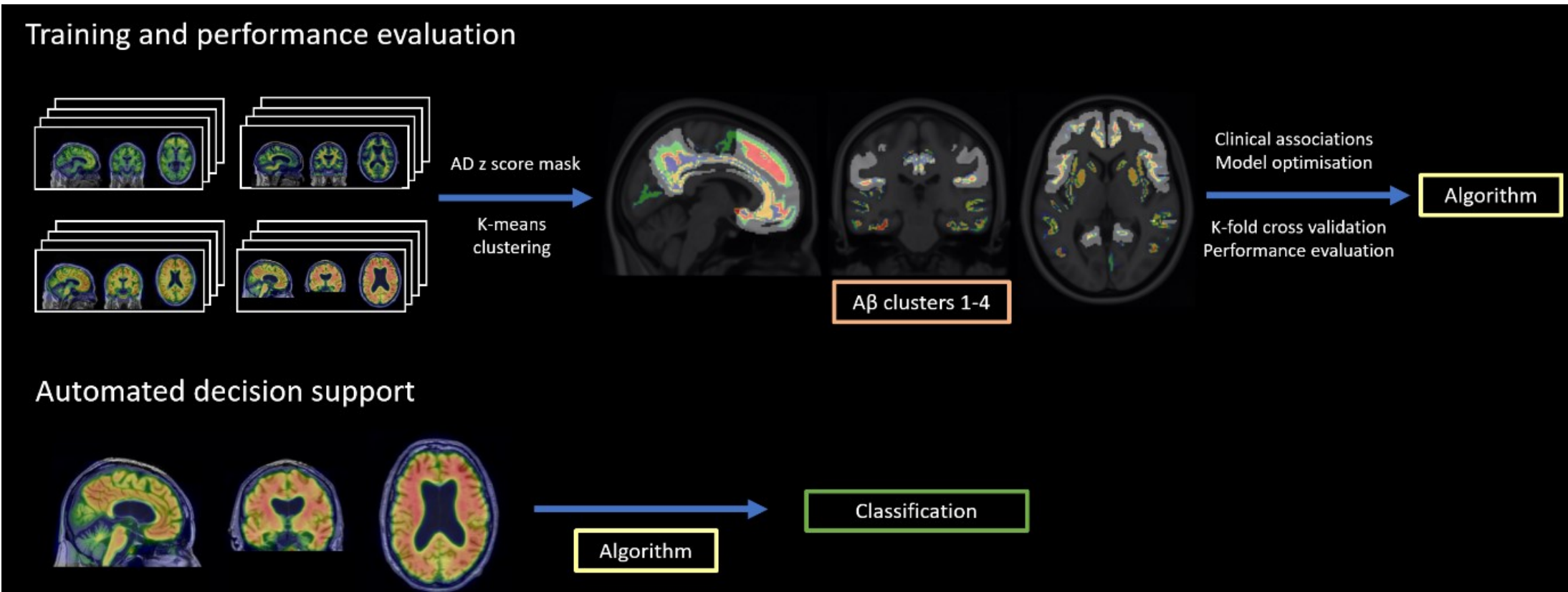
Materials and Methods

Study 1: Boosting the diagnostic power of amyloid- β PET using a data driven spatially-informed spatial classifier for decision support

A high-level overview of the methods used in the study is shown in figure 2.1 below.

Figure 2.1: Overview of study methods: Training and performance evaluation shows the input which is a combination of [^{18}F] Florbetapir PET and T1 structural MRI following co-registration, segmentation and registration to MNI 152 space stratified by group. This is used to create a binarized mask of the AD z-score voxels to focus where most variance of A β occurs in AD, and this is applied to the whole data set with k-means clustering to map cluster results to anatomy where A β clusters 1-4 are shown. Clinical associations, model testing and optimisation, and performance evaluation compared to other spatial methods are conducted to form an algorithm. This algorithm allows the input of a new [^{18}F] Florbetapir PET and T1 structural MRI and classifies the scan allowing for automated decision support.

Figure 2.1



Data

Data used in the preparation of this thesis were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). The ADNI was launched in 2003 as a public-private partnership, led by Principal Investigator Michael W. Weiner, MD. The primary goal of ADNI has been to test whether serial magnetic resonance imaging (MRI), positron emission tomography (PET), other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of mild cognitive impairment (MCI) and early Alzheimer's disease (AD). ADNI received US ethical approval from 58 study locations with all participants providing informed consent (ClinicalTrials.gov identifier: NCT01231971).

Participants

Participants from the ADNI database comprised of cognitively normal (CN), early mild cognitive impairment (EMCI), late mild cognitive impairment (LMCI) and Alzheimer's Disease (AD) with available [^{18}F]Florbetapir amyloid PET and corresponding T1 MPRAGE structural MRI scans (total $n=758$) and were aged 55-90 years old. They had to attend with an accompanying study partner, be able to speak English or Spanish fluently, and willing to undergo all study procedures including brain imaging and attend longitudinal follow up. Further exclusion

criteria included participants taking medication affecting cognitive function, Hachinski Ischaemic Score > 4, not on a stable dose of permitted medications 4 weeks before screening, Geriatric Depression Score < 6, visual and auditory acuity impairment that would affect neuropsychological testing, and women of child bearing potential. (123)

Participants were classified by into CN, MCI (early and late) and AD by clinical history and neuropsychological evaluation. CN participants had Mini Mental State Examination (MMSE) scores of 24-30 with no memory complaints and a Clinical Dementia Rating (CDR) of zero. MCI participants had MMSE scores of 24-30, CDR scores of 0.5 and objective memory impairment. AD patients had MMSE scores of 20-26 and CDR scores greater than 0.5.

Imaging Data

Each participant underwent a 20-minute [^{18}F]Florbetapir PET scan 50 minutes post-injection (370 ± 37 MBq) according to the standardised ADNI protocol. Image pre-processing steps are described elsewhere (adni.loni.usc.edu/methods/pet-analysis/pre-processing).

[^{18}F]Florbetapir PET scans were analysed using MIAKATTM (version 4.3.7, miakat.org), that implements MATLAB (version R2019a), FSL (version 5.0.4) functions for brain extraction and SPM12 (fil.ion.ucl.ac.uk/spm) for image

segmentation and registration. [¹⁸F]Florbetapir PET scans were nonlinearly registered into MNI152 space with DARTEL. Structural MRI images segmented into GM (grey matter) and WM (white matter) with SPM12 and registered to a group average template. The group average template was then registered to MNI152 space. Each participants' [¹⁸F]Florbetapir PET SUVr image was registered to the corresponding MRI using a rigid-body registration. Individuals' DARTEL flow field and template transformation was applied without modulation resulting in [¹⁸F]Florbetapir images in MNI152 space.

SUVr data were quantified by dividing each SUV image by its mean cerebellar GM reference. Mean and standard deviation voxel-wise GM masked images of each group (CN, EMCI, LMCI, AD) were created. Three Z-score GM masked image of each group (EMCI, LMCI and AD) relative to CN was created where the group z-score = (group mean – CN mean)/CN standard deviation). A de-noised binarised mask (at SUVr threshold 1.1 (124–126)) of the AD z-score voxels was created to focus where most variance of A β occurs in AD.

Cluster based method

The AD z-score thresholded mask was applied to the whole [¹⁸F]Florbetapir PET images dataset (all individual unthresholded GM masked images in MNI152). Voxels from 758 participants were clustered into k groups according to their SUVr

intensities. To achieve this all spatially constrained voxels for each participant were concatenated into a long vector and a one-dimensional k-means clustering of a 1000 iterations per voxel using MATLAB was performed across all participants between corresponding masked voxels for $k = 2-13$, where k denotes the number of clusters. For each voxel, the membership of the cluster it belongs to was determined, and the output clustering results were reshaped from a long vector into brain image space, mapping the clusters to anatomy.

In order to form clinical associations of each k cluster and its divisions with disease category, each group (CN, EMCI, LMCI, AD) GM masked mean image was constrained by the AD z-score binarised mask. For each k cluster output image, its divisions were created and saved as a mask ($n = 90$ masks for $k = 2-13$). Each k cluster division mask was applied to each group. GM masked mean image was constrained by the AD z-score binarised mask in order to focus on AD relevant spatial information.

Associations with diagnostic category and clinical variables

I had 4 clinically defined groups of participants and I considered them as defining a progression of disease in 4 stages (CN, EMCI, LMCI, and AD). I considered the representation of voxels in each cluster by clinical stage and counted the number of voxels in each group within each k cluster's divisions. This process of applying the

k division masks and counting positive voxels was also repeated for each individual participant, then grouped by diagnostic category and ordered by progression. An amyloid positive voxel was determined accordingly to commonly associated thresholds for positivity. (124,125) Additionally, spillover from WM was assessed by calculating the mean SUVR in individual participants within a WM mask and stratifying by group. MMSE and CDR correlations were explored for A β clusters 1-4 for scans from 730 participants where an MMSE was recorded within 6 months of the [^{18}F]Florbetapir PET and for 758 participant scans for which a CDR score was recorded within 3 months of [^{18}F]Florbetapir PET.

Classification and performance evaluation

In order to classify AD vs CN a support vector machine (SVM) was chosen. (127) An optimum SVM was selected by adjusting kernels, and serially testing classifications using quadratic, linear, and coarse Gaussian SVMs. 20-fold cross-validation using a quadratic SVM across 342 participants and 2 response variables (AD, CN) was performed in MATLAB. The optimised machine learning classifier for the following inputs was assessed for each individual participant for a total of 6 features amyloid PET positive voxel clusters number 1-4 (4), GM voxels positive, GM mean SUVR). The performance of all 6 features were compared to the *de novo* A β clusters 1-4 (4 features), and features of existing measures namely composite SUVR, and 2 features of GM voxels positive, GM mean SUVR using logistic

regression. Confusion matrices, accuracy, sensitivity, specificity, AUC-ROC, alongside error rates were evaluated for the 4 inputs.

Ethics approval and consent to participate

ADNI received US ethical approval from 58 study locations with all participants providing informed consent (ClinicalTrials.gov identifier: NCT01231971).

Availability of data and materials

The datasets analysed during the current study are available from the Alzheimer's Disease Neuroimaging Initiative (ADNI) <http://adni.loni.usc.edu/>

The datasets generated during the current study will be available in the github repository, <https://github.com/brainregion/>

Data collection and sharing for this project was funded by the Alzheimer's Disease Neuroimaging Initiative (ADNI) (National Institutes of Health Grant U01 AG024904) and DOD ADNI (Department of Defense award number W81XWH-12-2-0012). ADNI is funded by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, and through generous contributions from the following: AbbVie, Alzheimer's Association; Alzheimer's Drug Discovery Foundation; Araclon Biotech; BioClinica, Inc.; Biogen; Bristol-Myers Squibb Company; CereSpir, Inc.; Cogstate; Eisai Inc.; Elan

Pharmaceuticals, Inc.; Eli Lilly and Company; EuroImmun; F. Hoffmann-La Roche Ltd and its affiliated company Genentech, Inc.; Fujirebio; GE Healthcare; IXICO Ltd.; Janssen Alzheimer Immunotherapy Research & Development, LLC.; Johnson & Johnson Pharmaceutical Research & Development LLC.; Lumosity; Lundbeck; Merck & Co., Inc.; Meso Scale Diagnostics, LLC.; NeuroRx Research; Neurotrack Technologies; Novartis Pharmaceuticals Corporation; Pfizer Inc.; Piramal Imaging; Servier; Takeda Pharmaceutical Company; and Transition Therapeutics. The Canadian Institutes of Health Research is providing funds to support ADNI clinical sites in Canada. Private sector contributions are facilitated by the Foundation for the National Institutes of Health (www.fnih.org). The grantee organization is the Northern California Institute for Research and Education, and the study is coordinated by the Alzheimer's Therapeutic Research Institute at the University of Southern California. ADNI data are disseminated by the Laboratory for Neuro Imaging at the University of Southern California.

Study 2: Widespread cell stress and mitochondrial dysfunction in early Alzheimer's Disease

Study Design

I included 28 participants. Twelve patients with amnesic early AD were recruited for this study, and 16 controls were drawn from the MINDMAPS healthy volunteers cohort. Molecular Imaging of Neurodegeneration – Mitochondria, Associated Proteins, Synapses (MINDMAPS) is an academic/industry consortium to investigate neurodegenerative diseases using these PET targets (invicro.com/mind-maps). All participants underwent screening, [^{18}F]BCPP-EF, [^{11}C]UCB-J, [^{11}C]SA4503 dynamic PET imaging, (all PET within ~4 weeks), volumetric MRI and ASL scanning. All MR and PET measures were repeated in 8 of the AD patients 12-18 months after the baseline imaging.

AD patients met National Institute on Aging-Alzheimer's Association (NIA-AA) core clinical criteria for probable AD dementia, or amnesic mild cognitive impairment. They were beta-amyloid positive (based on [^{18}F]Florbetaben PET findings), were aged >50, had a Mini Mental State Examination (MMSE) score ≥ 18 , and had the capacity to give informed consent. Participants taking symptomatic pharmacotherapy (memantine) for AD memory were on a stable dose for at least 6 weeks prior to the baseline evaluation, and were willing to adhere to PET scan procedures.

Exclusion criteria for both groups included major psychiatric/neurological/ medical illness or infection, use of medication (e.g. donepezil, levetiracetam) known to either bind directly to SV2A, S1R or MC1, or impair cognition (daytime sedatives, pain medications or anticonvulsants), and prior radiation exposure >10mSv in the past year. Routine clinical blood samples alongside ApoE genotyping were performed.

Neuropsychological and cognitive assessments included the MMSE, Addenbrooke's Cognitive Exam (ACE-III), Repeatable Battery for Assessment of Neuropsychological Status (RBANS) and the National Adult Reading Test (NART). Specific RBANS tests included assessing immediate memory (list learning and story memory), visuospatial perception (figure copy and line orientation), language (picture naming and semantic fluency), attention (digit span and coding), and delayed memory (list recall, list recognition, story memory and figure recall). Specific ACE-III tests included rating attention (orientation), memory (word recall, address recall, semantic recall), fluency (word generation), language (sequenced commands, writing, repetition, pronunciation) and visuospatial (figure copy, clock drawing, dot counting, letter recognition).

All participants provided written informed consent. Screening and scanning were conducted at the Invicro imaging centre, Hammersmith Hospital Site, London. Ethical approval for this study was provided by the NHS London - Brighton &

Sussex Research Ethics Committee (REC 18/LO/0179) for AD patients and East of England Cambridge Central & South Research Ethics Committee for controls. Radiation safety was approved by the Administration of Radioactive Substances Advisory Committee (ARSAC R92). Local site approval was provided by Imperial College London Joint Research Office. Participants were recruited from Imperial College Healthcare NHS Trust, West London Mental Health Trust, and Cambridge University Hospitals.

This is the first study evaluating the effects of neurodegeneration on the three molecular targets (SV2A, MC1 and S1R) alongside MR measures such as structural, ASL, NODDI and DTI in Alzheimer's patients in vivo, and as such the sample size was based on feasibility. In pilot studies of individual radioligands at the time of design [^{18}F]BCPP-EF and [^{11}C]UCB-J conducted in the US and Japan, decreases of the order of 20% in MCI and 35% in AD patients have been observed in similar sample sizes (Carson, Tsukada – personal communications). The variability of the radioligands above ranges from 3-15% depending on the ligand and region evaluated. Hence we believe that we are justified in examining 12 individuals with early AD in our study.

Image Acquisition

[^{18}F]BCPP-EF, [^{11}C]UCB-J, and [^{11}C]SA4503 were synthesised and PET/CT images and arterial blood samples acquired as previously described

(74,105,107,128). [^{18}F]Florbetaben was purchased from Alliance Medical. Imaging was performed using either a Siemens Biograph 6 True Point (FWHM: 7.35 mm, 7.35 mm, 14.4 mm) or Siemens Biograph 6 HiRez (FWHM: 7.0 mm, 7.0 mm, 14.4 mm) PET/CT scanner. Both scanners are similar models with the same scanner used for each subject for all scans in the study to negate scanner differences affecting results. There was no significant differences between scanner results for test-retest in controls and within both AD patients and controls groups. Prior to each imaging session a low-dose CT scan was acquired to enable attenuation correction (30 mAs, 130 KeV, 0.55 pitch). AD patients received a [^{18}F]Florbetaben scan at screening, acquired over 20 min following a 90 min distribution phase. All participants received [^{18}F]BCPP-EF, [^{11}C]UCB-J and [^{11}C]SA4503 scans with the same camera which was also used for all follow-up scans for an individual. The PET tracers were administered as an intravenous bolus (20mls over 20 seconds), and blood samples were collected from the radial artery to assess changes in the concentration of radioactivity in whole blood and plasma, as well as the percent of unmetabolized tracer over the course of the PET scan radioactivity sample collection. Following tracer administration, dynamic emission data was acquired in list mode over 90 minutes and reconstructed into 26 time frames (frame durations: 8x15 s, 3x60 s, 5x120 s, 5x300 s, 5x600 s) using discrete inverse Fourier transform reconstruction with corrections for attenuation, randoms and scatter.

Table 2.1 Total potential maximum doses in mSv received by participants

PET RP	Maximal activity (MBq)	Maximal PET ED (mSv)	CT (mSv)	CTs per scan	Maximal CT ED (mSv)	Maximal ED per PETCT scan (mSv)	scans	Total ED (mSv)
UCB-J	300	1.01	0.36	2	0.72	1.7	2	3.5
SA4503	300	2.01	0.36	2	0.72	2.7	2	5.5
BCPP-EF	100	2.2	0.36	2	0.72	2.9	2	5.8
Florbetaben	100	1.93	0.36	2	0.72	2.7	1	2.7
Total							7	17.4

Thus, for the study as a whole, the maximal exposure for any subject will be 17.4 mSv with no more than 10 mSv in any 12 month period. This exposure will place the study in category IIb (intermediate to moderate risk) of the International Commission on Radiological Protection (ICRP), an international workgroup on radiological protection.

All MRIs were acquired with a Siemens 3T Trio clinical scanner (Siemens Healthineers, Erlangen, Germany) using a 32-channel phased-array head coil. T1-weighted structural data (a 3D MPRAGE sequence; TE = 2.98 ms, TR = 2300 ms, flip angle = 9°, voxel size = 1.0 mm x 1.0 mm x 1.0 mm) and multi-TI Pulsed ASL (5 TIs: 1200-2400ms with 300ms increments) are presented in this thesis. All measures were repeated 12-18 months later for the AD group.

Image Analysis and statistical analysis

[¹⁸F]Florbetaben data were quantified by Amyloid IQ, (129) using 33% as cut-off for amyloid positivity and inclusion in the study.

The dynamic PET image analysis pipeline, including generation of an arterial input function, tracer kinetic modelling, model comparison and selection and time stability assessment, has been described previously. (105) Briefly, PET scan emission data were processed using MIAKAT™ (version 4.3.24, miakat.org). MIAKAT™ is implemented using MATLAB (version R2016a; Mathworks) and makes use of SPM12 (Wellcome Trust Centre for Neuroimaging, fil.ion.ucl.ac.uk/spm) functions for image segmentation and registration. Individual participant structural MRIs underwent grey matter segmentation and rigid body co-registration to a standard reference space. (59) The template brain image and associated CIC neuroanatomical atlas (130) was then nonlinearly warped to the individual participant's MRI where the regions of interest (ROI) were defined and volumes of regions in mm³ were measured. A centrum semiovale (CS) ROI was also generated from the automated anatomic labelling template as defined previously for use as a non-specific reference region for [¹¹C]UCB-J. (74) PET images were registered to each participant's MRI and corrected for motion using frame-to-frame rigid-body registration. Regional time activity curves (TAC) were generated for each ROI.

For ASL processing, T1w data was initially processed using FSL (*fsl_anat*) to provide tissue segmentations and nonlinear mapping to standard MNI 152 space (fsl.fmrib.ox.ac.uk). (131) By inversion of this nonlinear transformation, and concatenation with the T1-to-ASL (M0) alignment using FSL (*flirt*, 6 DOF), the CIC atlas was nonlinearly aligned to the ASL data to provide native-space ROIs. After motion-correction of the ASL data using FSL's *mcflirt*, (132) and generation of the perfusion-weighted time-series, CBF and arterial arrival time (AAT) modelling was performed using FSL's *oxford_asl* tool.

Regional volumes of distribution (V_T) were calculated for each of the three radioligands using the one-tissue compartmental model (1TC) for [^{11}C]UCB-J, and the multilinear analysis 1 (MA1) model for [^{18}F]BCPP-EF and [^{11}C]SA4503 as described previously. (105) The outcome parameter chosen for [^{11}C]UCB-J and [^{18}F]BCPP-EF was the DVR_{CS} , with both using the centrum semiovale as a pseudo-reference region where

$$\text{DVR}_{CS} = \frac{V_T^{ROI}}{V_T^{CS}} - 1$$

with V_T^{ROI} being the V_T in the region of interest and V_T^{CS} being the V_T in the centrum semiovale, a white matter pseudo-reference region. The volume of distribution corrected for plasma fraction (VT/f_p) was chosen as the outcome parameter for [^{11}C]SA4503. Partial volume-corrected data were generated using the Muller-Gartner algorithm. (86)

Parametric images in MNI 152 space were expressed as z-scores to facilitate interpretation of findings across the three radiotracers. [^{11}C]SA4503 and [^{11}C]UCB-J parametric maps were generated with the one tissue reversible compartmental model, solved voxel-wise using the basis function method. (90) [^{18}F]BCPP-EF parametric maps were generated using the MA1 method solved at the voxel level, using the same settings as the region level analysis. Group cross-sectional data of AD patients compared to controls was computed as z-scores (AD patients mean – controls mean)/(controls standard deviation). Group longitudinal compared with baseline AD patient data were also computed as z-scores (AD patients longitudinal mean – AD patients baseline mean)/(AD patients baseline standard deviation).

An orthogonal regression by PCA at a voxel-level basis was used to explore the relationship between [^{18}F]BCPP-EF, [^{11}C]UCB-J, [^{11}C]SA4503 between groups by inputting mean parametric PET images of these tracers' voxels plotted in 3D space. A plane was fitted to the CN data using PCA to minimise the sum of squared errors. The equation of the fitted plane ($([x\ y\ z] - \text{mean } xyz) \cdot \text{normal} = 0$), mean and each point's perpendicular distance to plane (residuals) and sum of squared errors were calculated for each group. The original 3D data, plane and their projection to the control plane together were plotted. Points were labelled as outliers if greater than 2 SD from the mean perpendicular distance of an individual point to the plane intersection for CN. AD voxel outliers were defined

as greater than 2 SD from the mean perpendicular distance of an AD individual point to the CN plane intersection. These outliers, relative to CN were reshaped into brain image space and mapped to anatomy to show the relative differences of these tracers in AD from CN. The areas shown by the orthogonal regression by PCA were used to select the *a priori* regions to test across modalities for regional comparisons.

Comparison of regional target binding between patient and control groups was performed using a two-tailed, unpaired Student's t-test for cross-sectional data and a two-tailed, paired Student's t-test for longitudinal data across *a priori* selected regions (frontal lobe, parietal lobe, temporal lobe, cerebellum, hippocampus, parahippocampal gyrus, precuneus, dorsolateral prefrontal cortex, thalamus, caudate, and cingulate cortex). I did not correct for multiple comparisons owing to the small sample size and exploratory nature of this study; however, adjusted p-values using the False Discovery Rate are documented in the supplementary material of this chapter.

Pearson's *r* correlations were performed across modalities and groups between [¹¹C]UCB-J, [¹⁸F]BCPP-EF, and [¹¹C]SA4503 uptake, regional volumes and CBF. Associations between imaging findings, neuropsychological and cognitive performance variables were interrogated. Correlations between differences over time and neuropsychological and cognitive performance measures were also

explored with *a priori* hypotheses. All statistics were analysed and plotted using R software (R-project.org).

Study 3: Synaptic microstructure in vivo in early Alzheimer's Disease

Study Participants

12 patients with amnesic early AD were recruited for this study and 16 controls were drawn from the MINDMAPS healthy volunteers cohort. All participants underwent screening, [^{11}C]UCB-J dynamic PET imaging, volumetric and NODDI MRI scanning.

The same criteria as above (Study 2) were used for AD patient inclusion and exclusion criteria, neuropsychological and cognitive assessments, consent and ethical approval, image acquisition for [^{11}C]UCB-J and structural MR, and image and statistical PET analysis.

All MRIs were acquired using a Siemens 3T Trio clinical scanner (Siemens Healthineers, Erlangen, Germany) with a 32-channel phased-array head coil. T1-weighted structural data was acquired using a 3D MPRAGE sequence (TE = 2.98 ms, TR = 2300 ms, flip angle = 9°, voxel size = 1.0 mm x 1.0 mm x 1.0 mm). Diffusion Tensor Imaging (DTI) was acquired with 64 diffusion directions at b = 1000 plus six b = 0 scans interspersed, using 3 x acceleration (MB = 3), two runs to provide acquisition in both the positive and negative phase-encoding direction and TE = 88.0 ms, TR = 3010 ms and a nominal voxel size = 1.9×1.9×2.0 mm. Neurite Orientation Dispersion and Density Imaging (NODDI) was performed using 2-shells with 32 directions at b=700 (plus 4 b=0's) and 64 directions at

b=2000 (plus 8 b=0's) and TE = 101.8 ms, TR = 3340 ms, MB = 3 and a nominal voxel size 1.9×1.9×2.0 mm.

For DTI and NODDI processing, T1w data was initially processed using FSL (fsl_anat) to provide tissue segmentations for the individual participant's T1w image (fsl.fmrib.ox.ac.uk)²⁵. GM and WM PVE maps were thresholded at 0.5 and binarized to provide GM and WM definitions, respectively. The CIC Atlas 23 was nonlinearly aligned to the subject's native T1w image to provide native-space ROIs.

For DTI processing, I estimated the susceptibility-induced off-resonance field from B0s (with AP and PA phase encoding) using FSL's topup tool, followed by correction of geometric distortion in the full DTI acquisition using FSL's eddy tool. DTI parameters (FA, MD, L1, RD) were computed from eddy-corrected DTI data using FSL's dtifit tool. Finally, FSL's epi_reg tool provided the DTI-to-T1 alignment parameters, and the DTI parameter maps were transformed to the individual participant's T1w image.

For NODDI processing, the DTI-to-NODDI (b0) alignment parameters were computed using FSL's flirt tool, then geometric distortion correction of the NODDI data was performed using FSL's eddy tool (providing DTI-to-NODDI transformation parameters for fieldmap estimated in NODDI space). Estimation of NODDI parameters (ficvf (referred to here as NDI), ODI, FISO) from the eddy-corrected NODDI data was achieved with the University College London

Microstructure Imaging group NODDI MATLAB toolbox
(<http://mig.cs.ucl.ac.uk/index.php?n=Download.NODDI>). Finally, computation of NODDI-to-T1 alignment parameters used FSL's `epi_reg` tool and the NODDI parameter maps were aligned to the individual participant's T1w image.

Comparison of regional target binding between patient and control groups was performed using a two-tailed, unpaired Student's t-test for cross-sectional data and a two-tailed, paired Student's t-test for longitudinal data across *a priori* selected regions (frontal lobe, parietal lobe, temporal lobe, cerebellum, hippocampus, thalamus, caudate, anterior cingulate and posterior cingulate).

Pearson's *r* correlations were performed across modalities and groups for NODDI measures (NDI, ODI, FISO), [¹¹C]UCB-J and regional volumes. Group cross-sectional imaging data of AD patients compared to controls was computed as z-scores $(\text{AD patients mean} - \text{controls mean})/(\text{controls standard deviation})$. Associations between imaging findings, neuropsychological and cognitive performance variables were explored. Correlations between these measures and neuropsychological and cognitive performance measures also were explored for a priori hypothesis generation. Corrections for multiple comparisons were calculated using the false discovery rate and shown in supplementary tables. All statistics were analysed and plotted using R software (R-project.org).

Chapter 3

Boosting the diagnostic power of amyloid- β PET using a data driven spatially-informed classifier for decision support

Abstract: Amyloid- β ($A\beta$) PET has emerged as clinically useful for more accurate diagnosis of patients with cognitive decline. $A\beta$ deposition is a necessary cause or response to the cellular pathology of Alzheimer's Disease (AD). Usual clinical and research interpretation of amyloid PET does not fully utilise all information regarding the spatial distribution of signal. I present a data driven, spatially-informed classifier to boost the diagnostic power of amyloid PET in AD. Voxel-wise k-means clustering of amyloid-positive voxels was performed, clusters were mapped to brain anatomy, and tested for their associations by diagnostic category and disease severity with 758 amyloid PET scans from volunteers in the AD continuum from the Alzheimer's Disease Neuroimaging Initiative (ADNI). A machine learning approach based on this spatially constrained model using an optimised quadratic support vector machine was developed for automatic classification of scans for AD vs non-AD pathology. This classifier boosted the accuracy of classification of AD scans to 81% using the amyloid PET alone with an area under the curve (AUC) of 0.91 compared to other spatial methods. This increased sensitivity to detect AD by 15%, and the AUC by 9% compared to use of a composite region of interest SUVR. The diagnostic classification accuracy of

amyloid PET was improved using an automated data driven spatial classifier. My classifier highlights the importance of considering the spatial variation in A β PET signal for optimal interpretation of scans. The algorithm now is available to be evaluated prospectively as a tool for automated clinical decision support in research settings.

Background

Alzheimer's Disease (AD) is characterised by amyloid- β (A β) (23,37,38) and tau deposition (44) in the brain. This neuropathology progresses with disease symptoms and severity. AD affects 50 million people worldwide and, while it has no cure, its diagnosis has a major impact on people, their families and clinical care. (2) The number of people affected is forecast to triple by 2050 (5) making confident early diagnosis and monitoring ever more critically important.

A β deposition in the brain is a necessary element (20) in the development of AD. (93) Higher brain A β deposition is associated with faster memory decline (95) and regional hypometabolism in distally connected brain regions. (33) Amyloid PET in life correlates well with *post mortem* A β deposition. (13) Amyloid PET thus has emerged as clinically useful for the diagnosis of patients with cognitive decline. (96,97) Amyloid PET has also been used both to better ensure patient diagnoses for inclusion and as an endpoint measure in clinical trials. (100–102) Supplementation of clinical assessment with amyloid PET increases the accuracy of diagnosis and changes patient management. (14,97–99) However, one challenge is that

cognitively normal individuals and other non-AD pathologies also can show A β deposition (133,134) albeit with differential regional vulnerabilities.

The neuropathology in AD is distinguished by a neuroanatomically distinct pattern of A β and tau deposition, with a hypothesised mechanistic relationship between amyloid deposition and tau. (23,38,44) Early detection of pathological amyloid deposition may help to identify people at higher risk of progression to AD. Regional vulnerabilities to the accumulation of pathological proteins and neurodegeneration may arise as a consequence of their functional anatomy. Defining the changing regional distribution of the A β PET signal with progression thus may help both to characterise disease stages and understand their clinical expression. (135) More recently tau PET has been shown to be useful in discriminating AD pathology, however the high cost and low availability, and relative later life deposition, make it practically difficult at present. (136)

Most current clinical interpretation of amyloid PET in real-world settings relies on a visual read of scans. The trained interpreter delineates cortical grey-white matter differentiation of amyloid PET signal, where reduced/absent differentiation of tracer uptake indicates A β positivity. (137) A metric such as a global standardized uptake value ratio (SUVR) may be included in reports, but only limited spatial distribution information is used. Alternative quantitative metrics are available, but they do not explicitly take into account relationships between neuroanatomical variations in A β accumulation and the progression of cognitive impairment. (129)

Regional and whole-brain specific information by use of a composite cortical SUVR mask to assess global amyloid burden is the primary outcome measure in many studies. (138–140)

My ultimate goal was to develop an automated decision support to differentiate between AD and non-AD participants based on amyloid PET by utilising disease-relevant neuroanatomical (spatial) information. To do this I developed A β masks using a data driven methodology with amyloid PET data from the Alzheimer's Disease Neuroimaging Initiative (ADNI). The *de novo* A β cluster masks generated and clinical classifications of participants then were used to optimise a classifier to discriminate AD vs non-AD pathology compared to commonly used alternative spatial constraints. In addition, I used the *de novo* A β clusters to further understand the behaviour of A β and regional vulnerabilities to disease.

Results

Demographics

758 participants with available [^{18}F]Florbetapir PET scans for processing were included in the analysis. Demographic data are shown in Table 3.1.

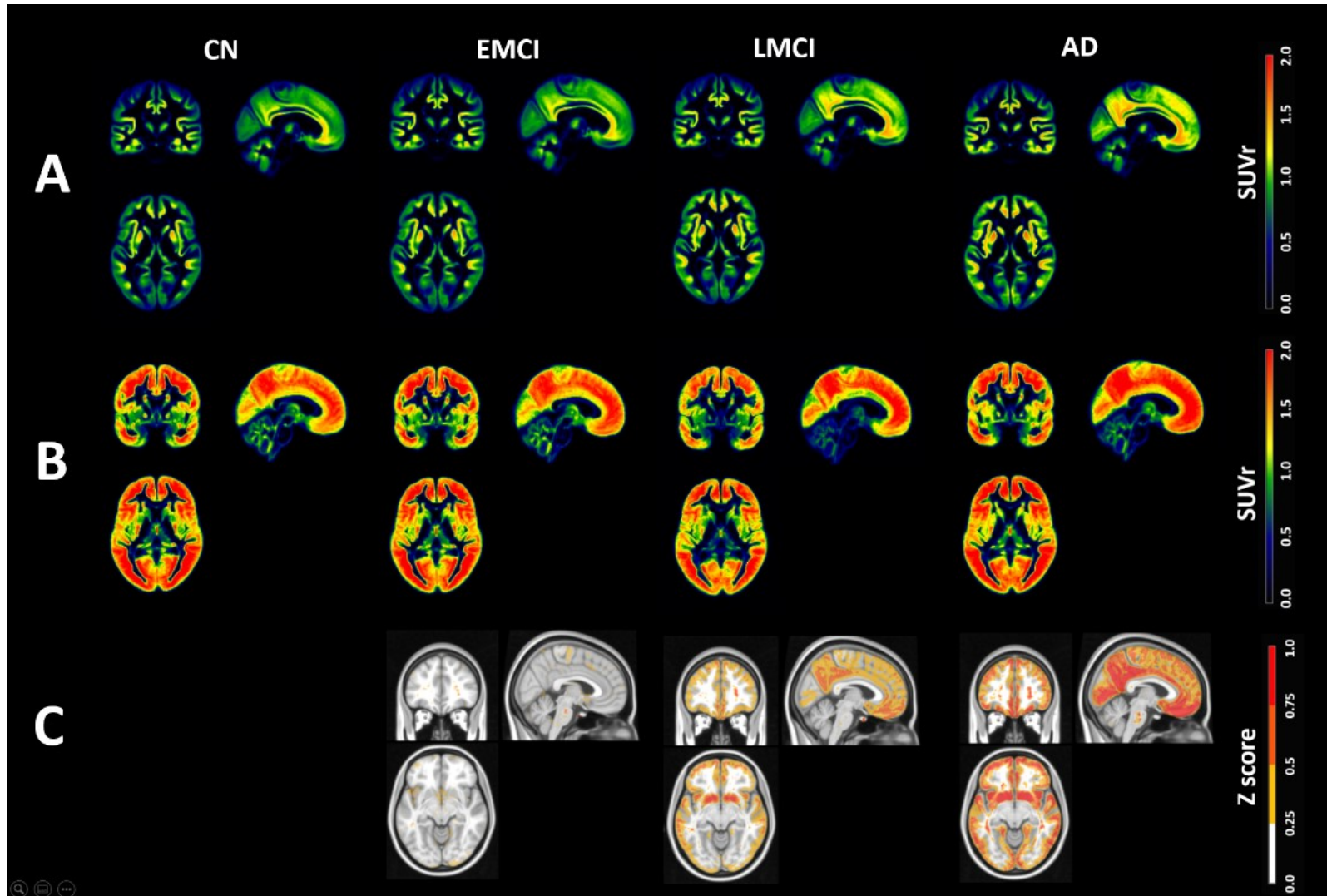
Table 3.1: Clinical demographics of participants

	CN	EMCI	LMCI	AD
n	199	196	198	165
Age (SD)	76(6)	70(7)	72(8)	76(8)
% male	54	57	49	55

Data driven cross sectional staging

Quantitative voxel-wise analysis across all scans showed greater uptake in the precuneus, posterior cingulate, isthmus cingulate, and the medial and lateral orbitofrontal cortices in EMCI. Greater uptake was found in lingual, pericalcarine, precentral and post-central cortices in LMCI and AD, (see Figure 3.1).

Figure 3.1: GM masked images showing the mean SUVr (panel A), standard deviation SUVr (panel B), and Z-score images overlaid on MNI 152 (panel C) and stratified by clinical group.



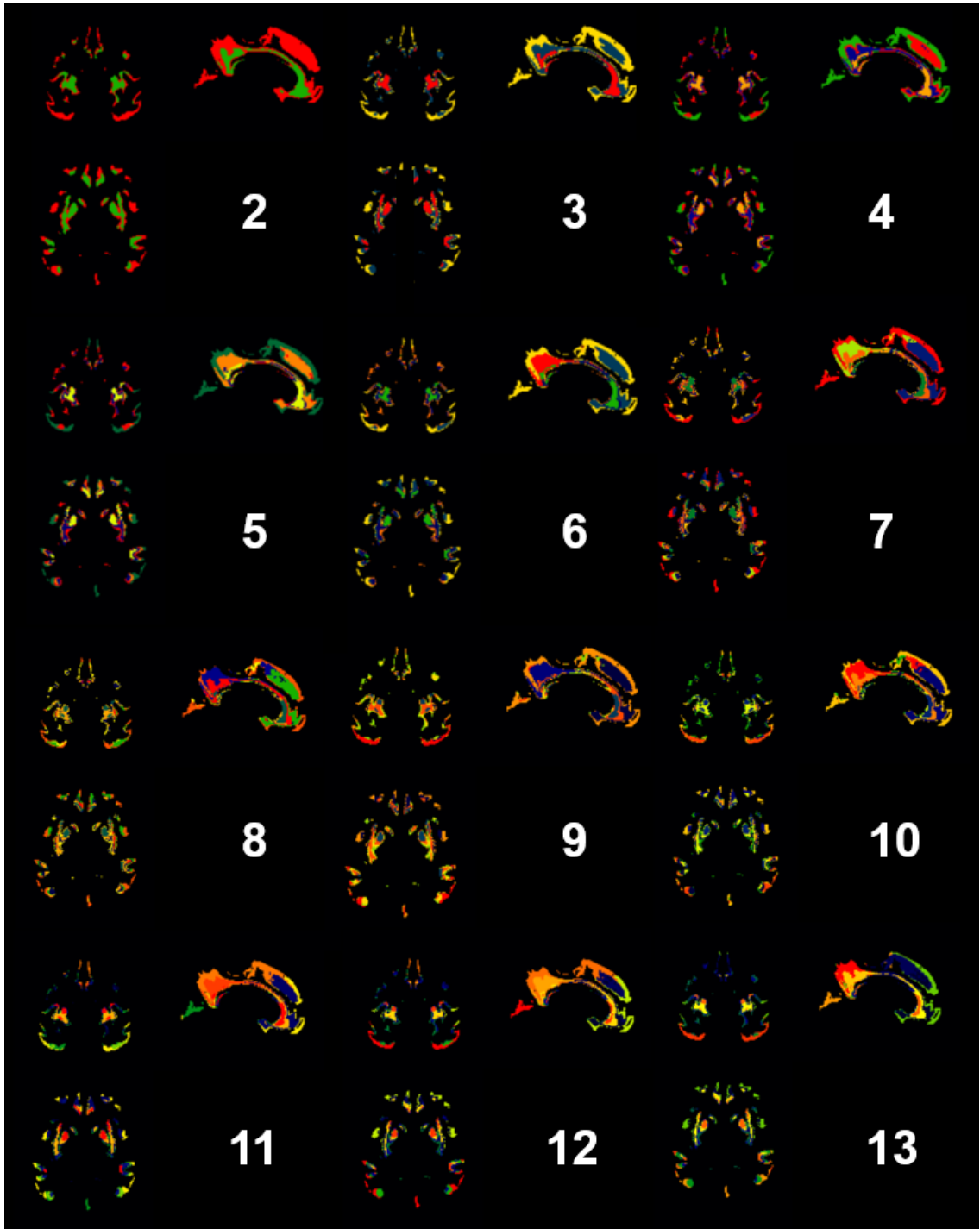
Z-score images were created of EMCI, LMCI and AD amyloid PET relative to CN. There are large increases in A β cluster expansion with clinical progression of disease in the above areas.

K-means results, and mapping clusters to anatomy

K-means clustering of amyloid PET positive voxels was performed and mapped to anatomy in order to form the classifier (see Figure 3.2). Areas that were delineated by clustering included the precuneus, subgenual area, thalamus and anterior and posterior cingulate, prefrontal, occipital, and orbitofrontal cortices.

A parsimonious model ($k = 4$) gave the most discriminative clusters for distinguishing the clinically defined groups of participants (see Figure 3.2), broadly consistent with expectations from cross-sectional pathological assessments *post mortem*. (141)

Figure 3.2: K means voxel-wise clustering results mapped to anatomy. Number corresponds to k value. Colours represent different clusters for a given k, and do not correspond between images when k varies.



Voxel clusters on silhouette plots for all k values tested and their discriminatory capacity are illustrated in Figures S3.1 and S3.2. The relationship of this spatial model with GM and composite regions of interest are shown in Figure 3.4. I also found that WM SUVR shows no difference between clinical groups (Figure S3.3), reducing the likelihood that spillover from WM contributes to cluster delineation.

Figure 3.3: Mean number of voxels of each group (GM masked mean constrained by AD z-score mask, thresholded at 1.1) for A β clusters 1-4 (A). A β clusters 1-4 map overlaid on MNI 152 (B)

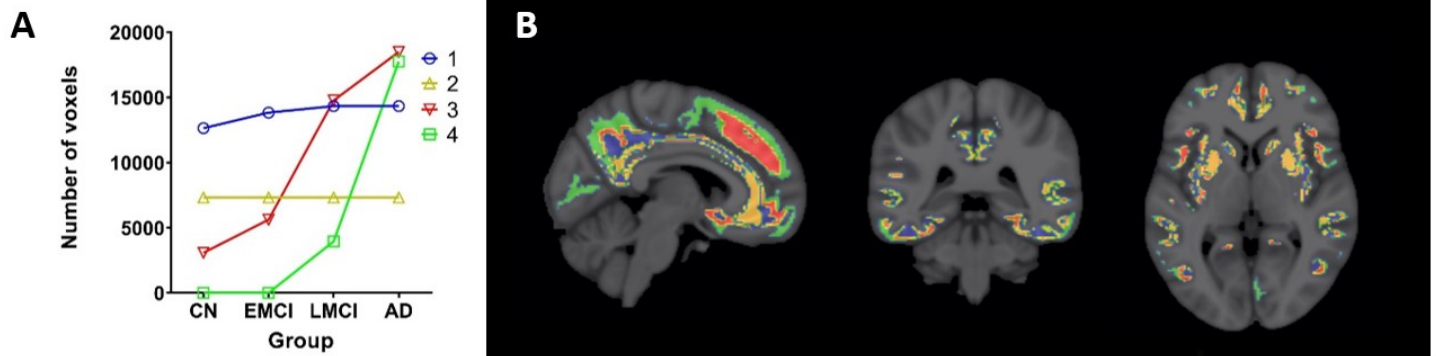
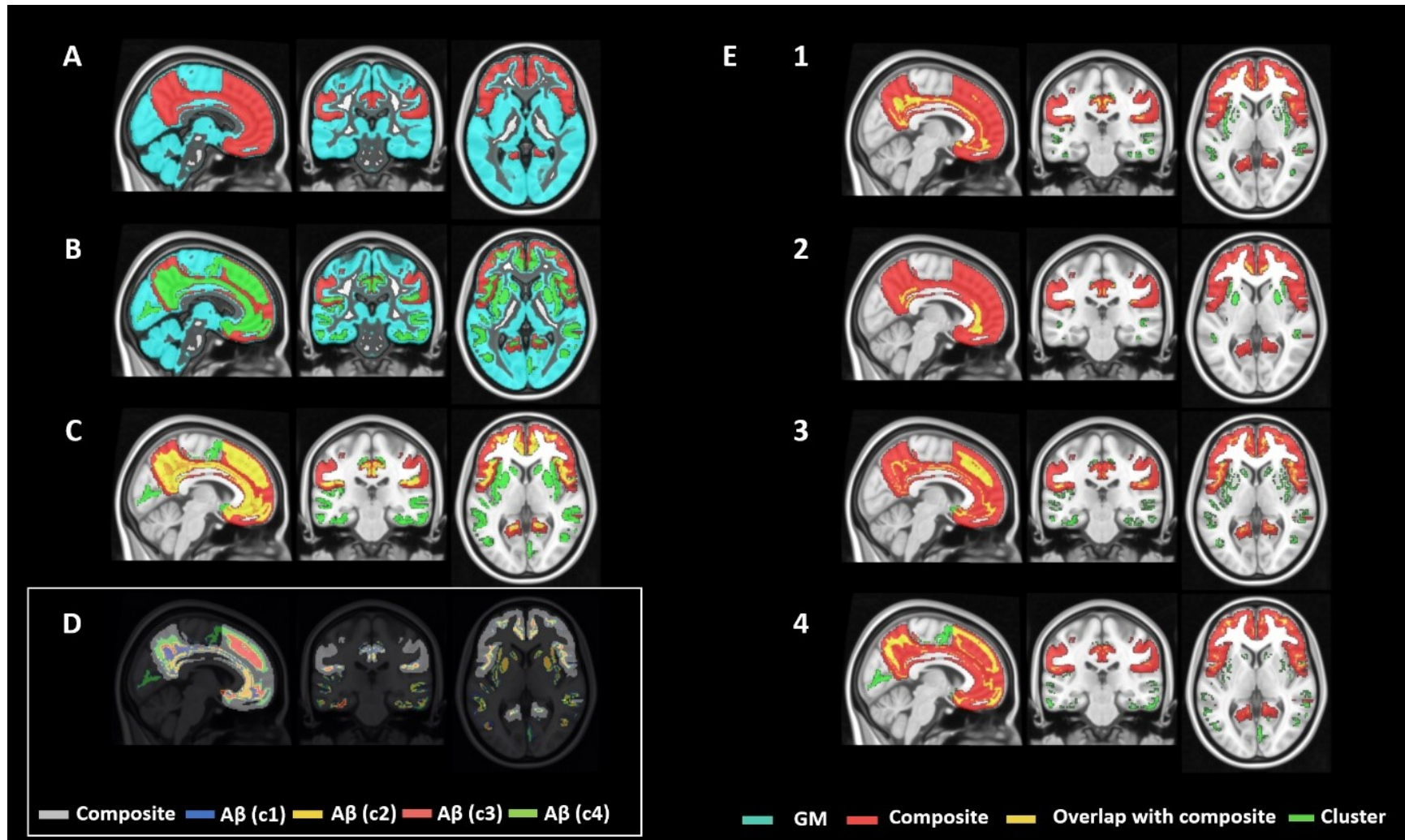


Figure 3.4: Overlays of composite region of interest, GM, and A β clusters 1-4 on MNI152. Panel A shows composite ROI on GM, panel B shows all A β clusters overlaid onto composite and GM. Panel D shows the relationship between A β clusters 1-4 and composite. Panel E shows the A β clusters 1-4 individually and the overlap with composite.



Clinical associations with amyloid PET positive clusters

Clinical associations of A β clusters defined using the $k = 4$ divisions with diagnostic category were explored (labelled as A β clusters 1-4). Violin plots were generated using A β clusters 1-4 masks applied to each individual in the whole population (Figure 3.5) and tabulated (Table 3.2).

Figure 3.5: Violin plots showing the distribution of number of positive amyloid voxels in each individual for A β clusters 1-4 stratified by clinical group. Dashed line indicates median value, dotted lines divide quartiles, and the width the probability density for $n = 758$ participants.

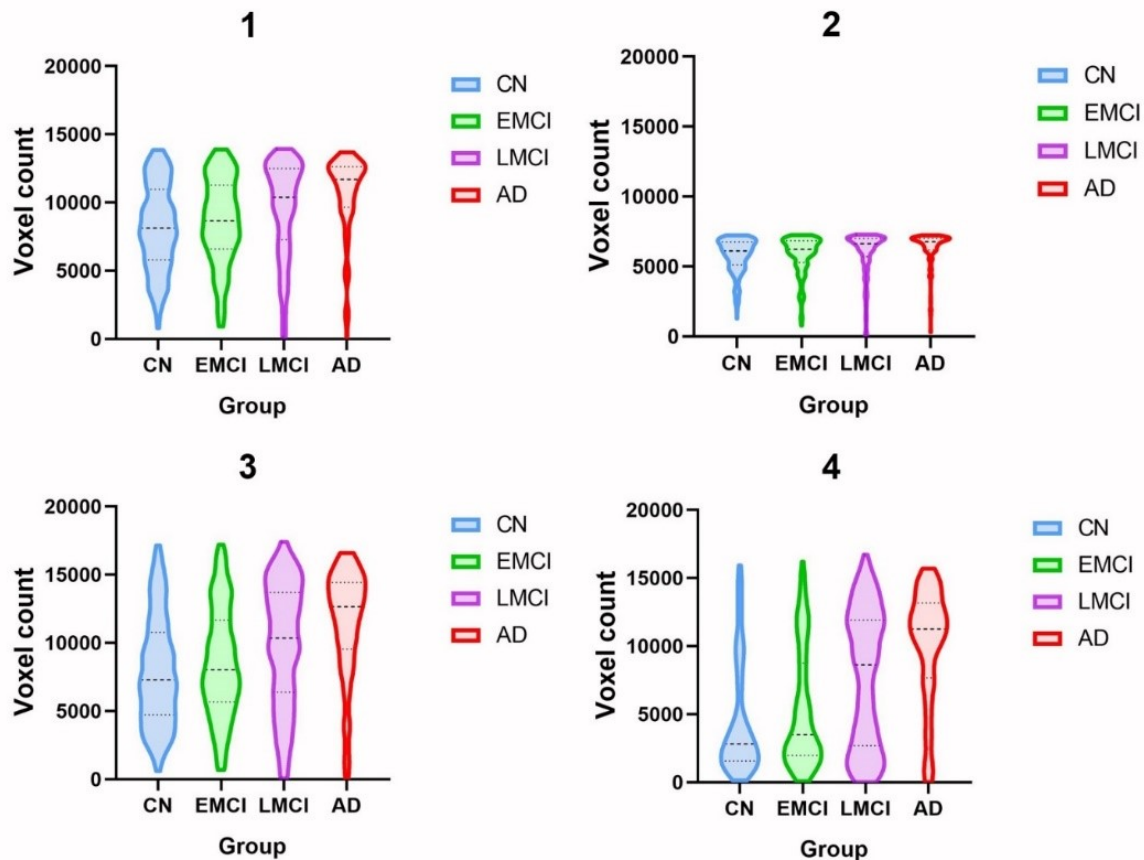


Table 3.2: Median, interquartile range (IQR) and skewness of A β positive voxels in clusters 1-4 across diagnostic category.

	CN Median (IQR)	EMCI Median (IQR)	LMCI Median (IQR)	AD Median (IQR)
1	8126 (5163)	8666 (4682)	10393 (5218)	11685 (2986)
2	6107 (1652)	6229 (1543)	6631 (1292)	6780 (829)
3	7285 (6038)	8028 (5988)	10350 (7309)	12666 (4908)
4	2818 (5370)	3492 (6797)	8633 (9214)	11264 (5498)
	Skewness g_1	Skewness g_1	Skewness g_1	Skewness g_1
1	-0.08	-0.37	-0.89	-1.79
2	-1.23	-1.62	-2.07	-2.71
3	0.40	0.20	-0.39	-1.10
4	1.21	0.83	-0.08	-1.01

Cluster 1 showed an increase in the number of A β positive voxels with increasing clinical severity. Both CN and EMCI had similar medians with a normally distributed spread across the population. LMCI and AD showed similar distributions with a skew towards higher voxels positive, and a long thin tail towards 0 (AD $g_1 = -1.79$). Cluster 2 had a similar distribution but occupied a smaller area.

Cluster 3 showed a normal distribution in the population of CN and EMCI. LMCI also showed a large spread in A β positive voxels across this population with a greater skew

towards more positive voxels. The AD group showed a greater skew towards positive voxels (AD $g_1 = -1.10$).

Associations of cluster 4 were markedly different from those of other clusters. CN and EMCI groups within this cluster had a skew towards 0 with a long thin tail extending towards higher voxels positive (CN $g_1 = 1.21$). LMCI scans showed a bimodal distribution with 2 peaks, one similar to the CN and EMCI groups at the lower end and a second with a similar distribution to AD at the higher end.

Taken together, these results highlight differences in distribution of A β PET signal clusters 1-4 stratified by clinical group. The clinically heterogeneous MCI populations shows a bimodal distribution (either like CN or AD) for cluster 4.

I evaluated the relationships of A β PET signal in clusters 1-4 with Mini-Mental State Examination (MMSE) and Clinical Disease Rating (CDR) scores. I found a negative relationship between signal in all of the A β clusters 1-4 and MMSE (Pearson's r values of -0.24, -0.14, -0.25, -0.36, respectively, $p < 0.001$) for 730 individuals where MMSE was documented within 6 months of amyloid PET.

I also found a positive relationship between A β clusters 1-4 and CDR score (Pearson's r values of 0.20, 0.10, 0.22, 0.32, respectively, $p < 0.001-0.004$) for 758 individuals where CDR was documented within 3 months of A β PET.

Optimisation of classifier and performance

A quadratic support vector machine (SVM) (127) was chosen as a classifier following optimisation for a combination of multiple features. An optimised linear regression model was chosen as the most accurate classification of single feature composite SUVR and for two features (GM voxels and GM SUVR).

The resultant classifier increased sensitivity by 15%, and the area under the receiver operating curve (AUC) by 9% compared to the “gold standard” composite SUVR. The classifier increased sensitivity by 39%, and the AUC by 20% compared to using the number of amyloid GM voxels positive (thereby adding spatial information) and the unconstrained amyloid GM SUVR (see Tables 3.3 and 3.4).

Table 3.3: Classification results comparing sensitivity, specificity, accuracy, AUC-ROC and error rates for different methods.

Method	Features	Sensitivity	Specificity	Accuracy	AUC	Error rate
A β clusters 1-4 , GM voxels and GM SUVR	6	0.83	0.81	0.81	0.91	0.19
A β clusters 1-4	4	0.74	0.84	0.80	0.87	0.20
Composite SUVR	1	0.67	0.86	0.79	0.82	0.21
GM voxels and GM SUVR	2	0.44	0.85	0.69	0.71	0.31

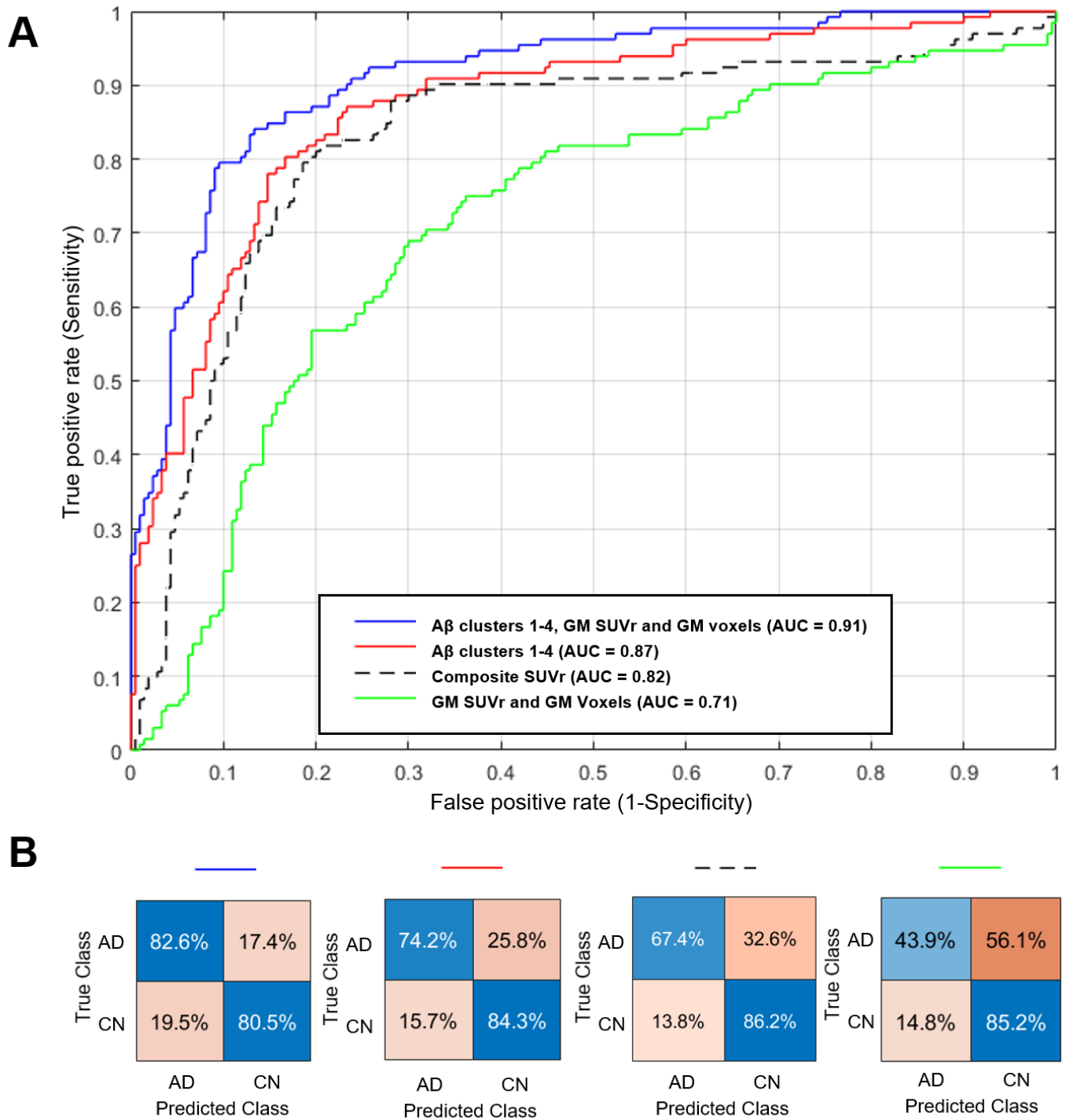
Table 3.4: Percentage change compared to the proposed method - 6 features (A β clusters 1-4 and GM voxels and GM SUVR)

Method	Sensitivity	Specificity	AUC
A β clusters 1-4	-8.3	3.0	-4.0
Composite SUVR	-15.2	4.9	-9.1
GM voxels and GM SUVR	-38.6	4.0	-19.8

The optimum combination of 6 features (A β clusters 1-4 and GM voxels and GM SUVR) boosted the accuracy of the classifier to classify AD to 81% using amyloid PET alone, with the highest sensitivity (0.83) and lowest error rate (AUC 0.91) relative to all other methods (see Figure 3.6).

Figure 3.6: AUC-ROC curve diagram of classification of AD vs CN for A β clusters 1-4 compared to and combined with other methods (A). The blue ROC curve is created by a combination of 6 features (A β clusters 1-4, GM voxels positive and GM mean SUVR) using SVM. The red ROC curve is created by the *de novo* A β clusters 1-4 using SVM. The dashed line ROC curve is a composite SUVR, and the green line is 2 features of GM voxels positive and GM mean SUVR using linear regression. Confusion matrices (B) with percentages for the corresponding ROC curves are on the right panel. These show the percentage of AD and CN individuals correctly predicted (blue), and incorrectly predicted (orange) for each method.

Figure 3.6



Discussion

I have generated a data driven algorithm that provides an automated classification of amyloid PET scans from people across the AD continuum that outperforms existing spatial measures tested here to boost the independent diagnostic power of amyloid PET. This automated classifier potentially could enhance the efficiency and accuracy with which amyloid PET scans are interpreted by radiologists and clinicians in the context of cognitive decline.

My approach, derived from a data driven model of amyloid PET positive clusters was optimised diagnostically and used to generate a classification system. In doing so, I improved the spatial sensitivity of amyloid PET to detect AD to 0.83, with a high AUC of 0.91 using an optimised support vector machine. Use of a k-fold cross-validation prevented the overfitting associated with hold out validation and is powerful as trained over a large number of amyloid scans. The classifier was trained using data from across multiple sites to capture variances typical for multi-centre and multi-scanner datasets across the AD continuum. The algorithm will be available online (github.com/brainregion) for evaluation as a tool for automated clinical decision support in research and as an adjunct to practice.

In developing my classifier, I had the opportunity to explore disease staging by mapping the trajectory of *in vivo* A β cluster expansion in AD using data driven clustering of amyloid PET. *Post mortem* evidence shows that A β plaque deposition spreads neocortically, moves to allocortical areas, then diencephalic nuclei, and finally cerebellum, (30) with associated patterns of neurofibrillary tau tangle deposition. (30,44) Newer *in vivo* PET approaches to assess fibrillar A β -beta have been useful for assessing *in vivo* pathology, however staging systems have either attempted to replicate classification systems used for *post mortem* neuropathology or are limited by the constraints of atlas based approaches. (142,143) My approach differed from traditional approaches and uniquely assessed voxels positive within novel A β clusters, as well as having an AD training set that is specific to A β pathology in AD. The A β clusters of a constrained area yielded higher accuracy, compared to a larger composite ROI (138,139,144) (the primary outcome measure in many studies) or whole GM. Enlargement, rather than absolute intensity, is able to evaluate unique properties of A β accumulation through cluster growth.

There are several advantages of using an “explainable” model rather than “black-box” approaches of deep-learning methods used previously for similar classification questions. (145–147) Foremost amongst these are the ease of interpretability of the spatial features derived from clustering and its relative simplicity of implementation. It also facilitates assessment of the relationships of measures to well-described pathological and clinical stages of disease. Other approaches combine different

amyloid tracers, (146) are mixed with other PET targets to achieve higher accuracy, (147) or focus on visually equivocal cases (145) and use the standard cortical SUVR mask, which my spatial model outperforms.

By exploring the A β clusters' clinical associations I found that a parsimonious model distinguishing 4 clusters was able to distinguish early from late disease and show differences in the distribution of positive A β voxels between disease groups. Usual clinical “visual” reads do not support this level of discrimination, which can be important for research applications, as well as having clinical relevance. This has relevance in that there may also be value in using different parts of the brain to track progression of disease – for example A β cluster 4 to evaluate track LMCI progression given the bimodal distribution, and understanding the regional vulnerability or inflexion point at which MCI converts to AD. Previous literature has evaluated A β in MCI but on smaller numbers. (148) This result may also reflect the diagnostic uncertainty of an MCI diagnosis, of which other causes need to be rule out, and the utility of A β PET to change management in an MCI population. (97) Future work involves testing the utility of using different cluster regions to assess change at different clinical severity timepoints in response to potential disease modifying therapeutics for AD in clinical trials.

One limitation of this study is that it derives data inferring progression based on a cross-sectional research derived dataset with A β PET signal accompanying MRI. However, over the long prodromal period of sporadic late onset AD, cross-sectional analyses have been shown to predict longitudinal change well. (126,149) Future work will involve replication in other cohorts to understand the extent to which results from these research-domain data can be generalised to usual clinical imaging. Other limitations include that I uniquely looked at expansion of clusters over a cut-point with data driven clusters and not the change in intensity at a voxel-wise basis. An advantage of my approach is that traditional SUVR analyses are not able to pick up expansions in volume of amyloid clusters. Most larger cohort studies in AD lack histopathologically confirmed ground truth diagnoses. This is a limitation to the accuracy of the classifier, however the large number of scans trained across multiple sites may help reduce the impact site-specific inaccuracies. The radiotracer for scans employed in my study is [^{18}F]Florbetapir, which is one of the most widely used amyloid tracers. The binding site appears to be similar to that of other A β tracers. (150) I do not expect quantitative differences with other A β tracers (125) but this would need further testing.

Accurate diagnosis of AD in clinic is difficult given the variety of neuropsychiatric symptoms, the varying trajectories of these, and confounding non-AD effects on cognition. I have developed a classifier that promises to boost the diagnostic power of amyloid- β PET using a data driven model of amyloid cluster progression with AD. This algorithm, which is based on the progression of regional brain vulnerabilities to AD,

provides automated diagnostic decision support that outperforms classifiers proposed previously for amyloid- β PET based on spatial constraints. The algorithm and its *de novo* cluster masks may be helpful for clinicians and radiologists investigating cognition in clinic.

Supplementary Material

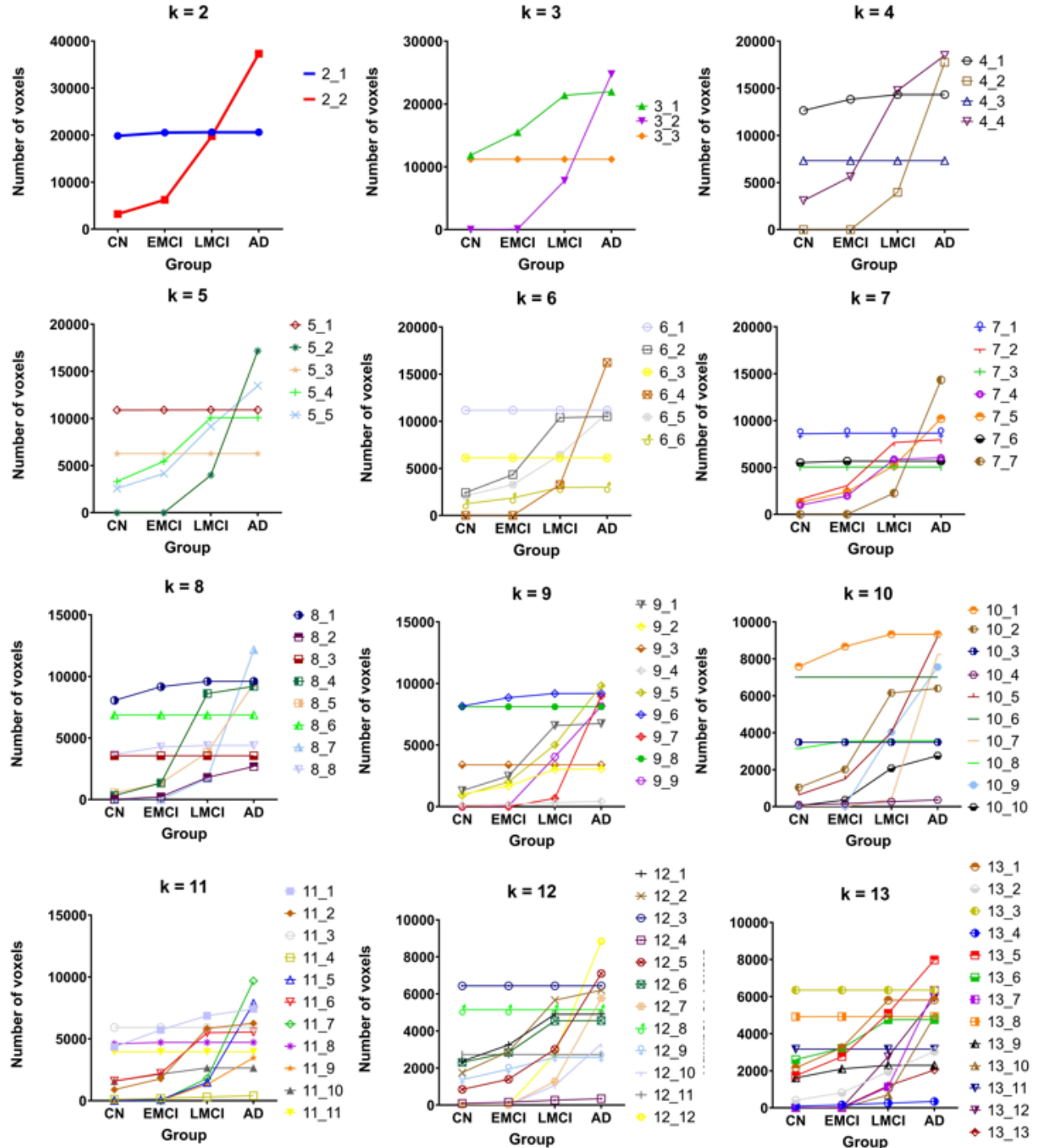
Figure S3.1: Number of voxels of each group (GM masked mean*ADzmap mask, thresholded at 1.1) within each k division.

Figure S3.2: Silhouette plots for 57929 voxels across 758 participants showing how close each voxel is in one cluster to voxels in neighbouring clusters.

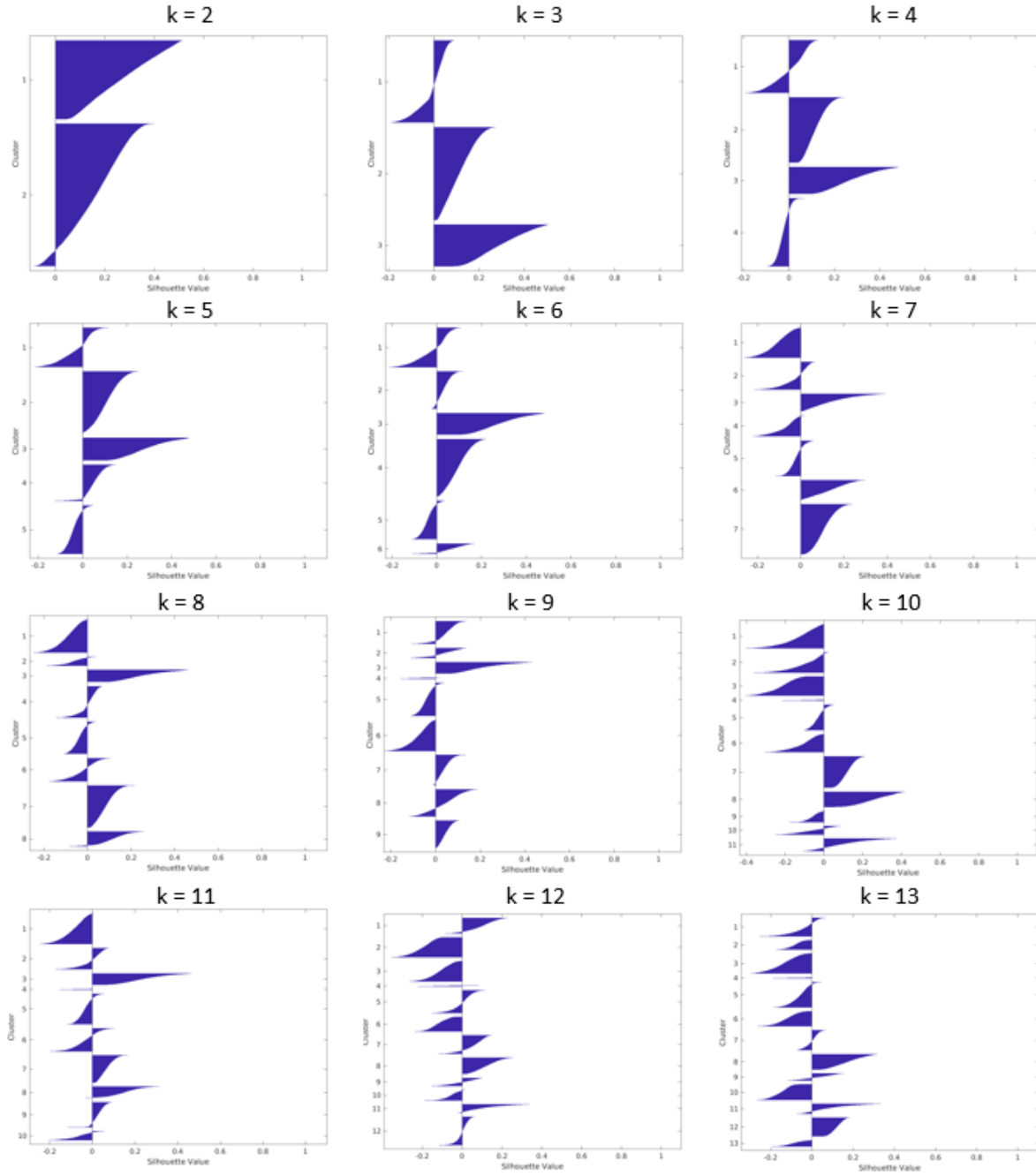
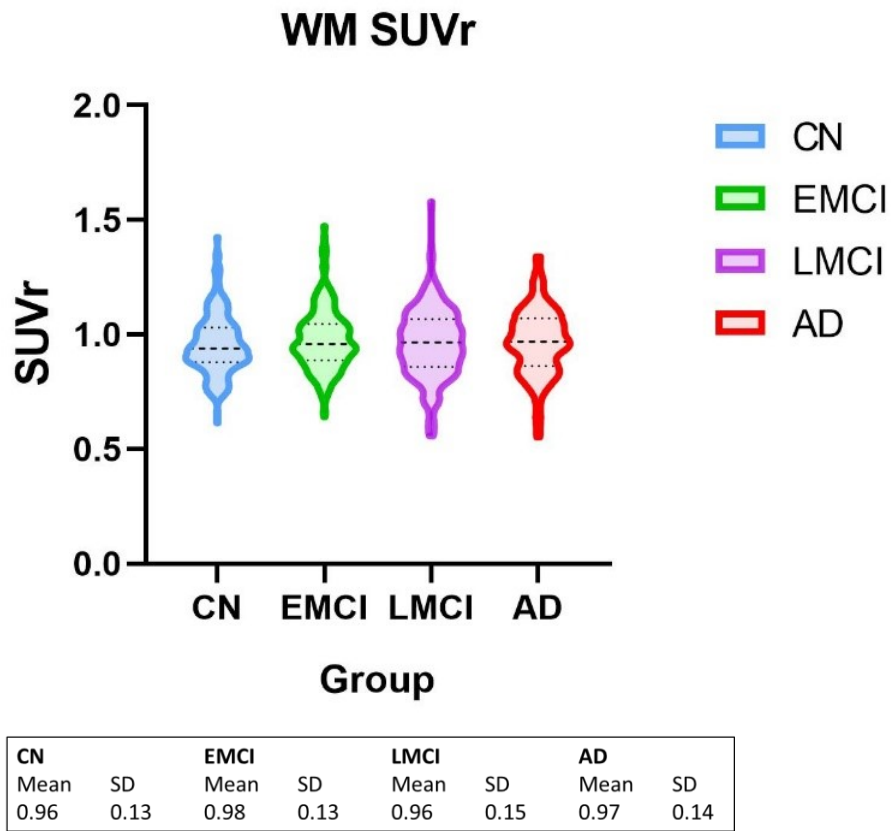


Figure S3.3: WM SUVR shows no difference between clinical groups.



Chapter 4

Widespread cell stress and mitochondrial dysfunction in early Alzheimer's Disease

Abstract: Cell stress and impaired oxidative phosphorylation are central to mechanisms of synaptic loss and neurodegeneration in the cellular pathology of Alzheimer's disease (AD). I quantified the *in vivo* density of the endoplasmic reticulum stress marker, the sigma 1 receptor (S1R) using [^{11}C]SA4503 PET, as well as that of mitochondrial complex I (MC1) with [^{18}F]BCPP-EF and the pre-synaptic vesicular protein SV2A with [^{11}C]UCB-J in 12 patients with early AD and in 16 cognitively normal controls. I integrated these molecular measures with assessments of regional brain volumes and brain perfusion (CBF) measured with MRI arterial spin labelling. 8 AD patients were followed longitudinally to estimate rates of change with disease progression over 12-18 months. The AD patients showed widespread increases in S1R ($\leq 27\%$) and regional decreases in MC1 ($\geq -28\%$), SV2A ($\geq -25\%$), brain volume ($\geq -23\%$), and CBF ($\geq -26\%$). [^{18}F]BCPP-EF PET MC1 density ($\geq -12\%$) and brain volumes ($\geq -5\%$) were further reduced at follow up in brain regions consistent with the differences between AD patients and controls at baseline. Exploratory

analyses showing associations of MC1, SV2A and S1R density with cognitive changes at baseline and longitudinally with AD, but not in controls, suggested a loss of metabolic functional reserve with disease. My study thus provides novel *in vivo* evidence for widespread cellular stress and bioenergetic abnormalities in early AD and that they may be clinically meaningful.

Introduction

Sporadic, late-onset Alzheimer's Disease (AD) is associated with progressive cognitive, social and functional decline after a long prodromal cellular phase. (50) The cellular phase is characterised neuropathologically by amyloid- β (A β) (23,37,38) and tau deposition and synaptic loss. The role of increased oxidative stress and an unfolded protein response (UPR) have been highlighted by biochemical and transcriptomic data, as well as genetic and biochemical evidence for early lipid dyshomeostasis. (53)

Multiple factors contribute to cell stress in AD. Variants in genes encoding proteins involved in lipid processing, phagocytosis and inflammation are associated with a genetic risk for AD. These proteins are ubiquitous throughout the brain, and biochemical and transcriptomic investigations suggest that dysfunction or dysregulation of these pathways all contribute to cell stress. (54) The sigma 1 receptor (S1R) is upregulated in the endoplasmic reticulum (ER) at sites of cell stress caused by

impaired mitochondrial oxidative phosphorylation. (55,56) Oxidative stress is a major pathogenic mechanism in AD, (57) although ER stress (e.g., from protein misfolding) may also contribute. (58)

A striking feature of early AD is the relatively focal nature of major pathological structural and functional changes, (29–32) despite common expression of genetic determinants of susceptibility throughout the brain. This focal appearance has been interpreted as a reflection of a selective vulnerability of those regions, determined by factors such as their connectivities, functional activities or metabolism. (33,34) Although not implicated in genetic susceptibility to AD and therefore unlikely to be causal, mitochondrial dysfunction and reduced oxidative phosphorylation have long been recognised in AD. (35,36) Amyloid- β toxicity and impaired autophagy/mitophagy are amongst the mechanisms hypothesised to contribute to this. Synaptic loss, which has a mechanistically proximate relationship to neuronal circuit dysfunction and cognitive symptoms, is another secondary pathology related both to amyloid- β toxicity, as well as to inflammatory glial activation. (45)

The long prodromal phase of AD pathology suggests that the healthy brain has considerable functional reserve. (82) Functional reserve can be defined operationally as the extent to which redundancy or plasticity allows normal cognition to be

maintained despite an impairment of functions (e.g., metabolic, synaptic or neuronal circuit) necessary to support it. Metabolic reserve can be defined as the level to which a metabolic process (such as oxidative phosphorylation) can be reduced before becoming rate limiting, e.g., for performance on standard cognitive tests. Synaptic reserve can be defined similarly in terms of the loss in synapse density in functionally relevant brain regions that can occur without associated impairment of related cognitive performance. Identification of pathological processes affecting pathways without sufficient functional reserve supports prioritisation of those pathways as therapeutic targets for AD, as the correlation of function and cognitive performance suggests that their modulation could lead to measurable clinical benefits.

I sought to characterise relationships between brain cell stress responses, oxidative metabolism and synaptic density in 12 patients with early AD in comparison with 16 healthy controls by imaging with three PET radiotracers to quantify the regional density of relevant molecular effectors. I used [^{11}C]SA4503 to image the sigma-1-receptor, [^{18}F]BCPP-EF to image mitochondrial complex I, and [^{11}C]UCB-J to image the synaptic vesicle glycoprotein 2A (SV2A) protein. Arterial spin labelling (ASL) (80) was also performed to quantify brain perfusion along with MRI volumetric assessments. AD patients were followed up 12-18 months later to evaluate the longitudinal change in these markers. By relating these markers of pathology to

cognitive performance, I was able to explore bioenergetic and synaptic functional reserve in the AD patients relative to the cognitively normal controls.

Results

Patient demographic and neuropsychological results

12 participants had a diagnosis of sporadic onset amnesic, PET amyloid positive early AD and 16 were cognitively normal controls. The two groups had equal numbers of females and males and similar premorbid intelligence levels as assessed by the NART. As expected, the AD patients had a significantly lower MMSE, immediate memory, language and delayed memory test scores compared to the control group but showed no impairment of visuospatial function (Table 4.1).

Table 4.1. Cross-sectional demographic and neuropsychological testing data for AD patients and cognitively normal controls at baseline

	AD (n=12)		CN (n=16)		p-value ¹
	Mean	SD	Mean	SD	
Age (Years)	75.50	7.82	65.88	9.82	0.008
MMSE	24.33	3.45	28.75	1.06	0.001
Immediate Memory	79.75	23.08	105.62	11.12	0.003
Visuospatial	101.83	17.54	98.56	9.61	0.568
Language	82.67	11.80	103.31	11.39	0.000
Attention	94.58	19.75	106.88	11.06	0.070
Delayed Memory	65.92	26.66	102.38	10.46	0.001
NART	36.67	8.63	35.94	10.85	0.845
¹ Unpaired two-tailed t-test assuming unequal variance					

Four AD patients did not complete their longitudinal follow-up scans due to progression of AD such that they could not comply with study requirements, new medical illnesses or excessive head motion during scanning. One participant started donepezil, which has high affinity for the S1R, so was excluded from the follow-up

[¹¹C]SA4503 PET scan. In summary, eight participants with early AD were able to complete both baseline and 12-18 month follow-up SV2A and MC1 PET and neuropsychological testing, and seven participants had both baseline and follow-up S1R PET scans. Ten participants completed baseline and follow up MRI scans. Reductions in ACE memory scores were found in those AD patients assessed longitudinally (Table 4.2).

Table 4.2. Neuropsychological test results for AD patients after 12-18 months

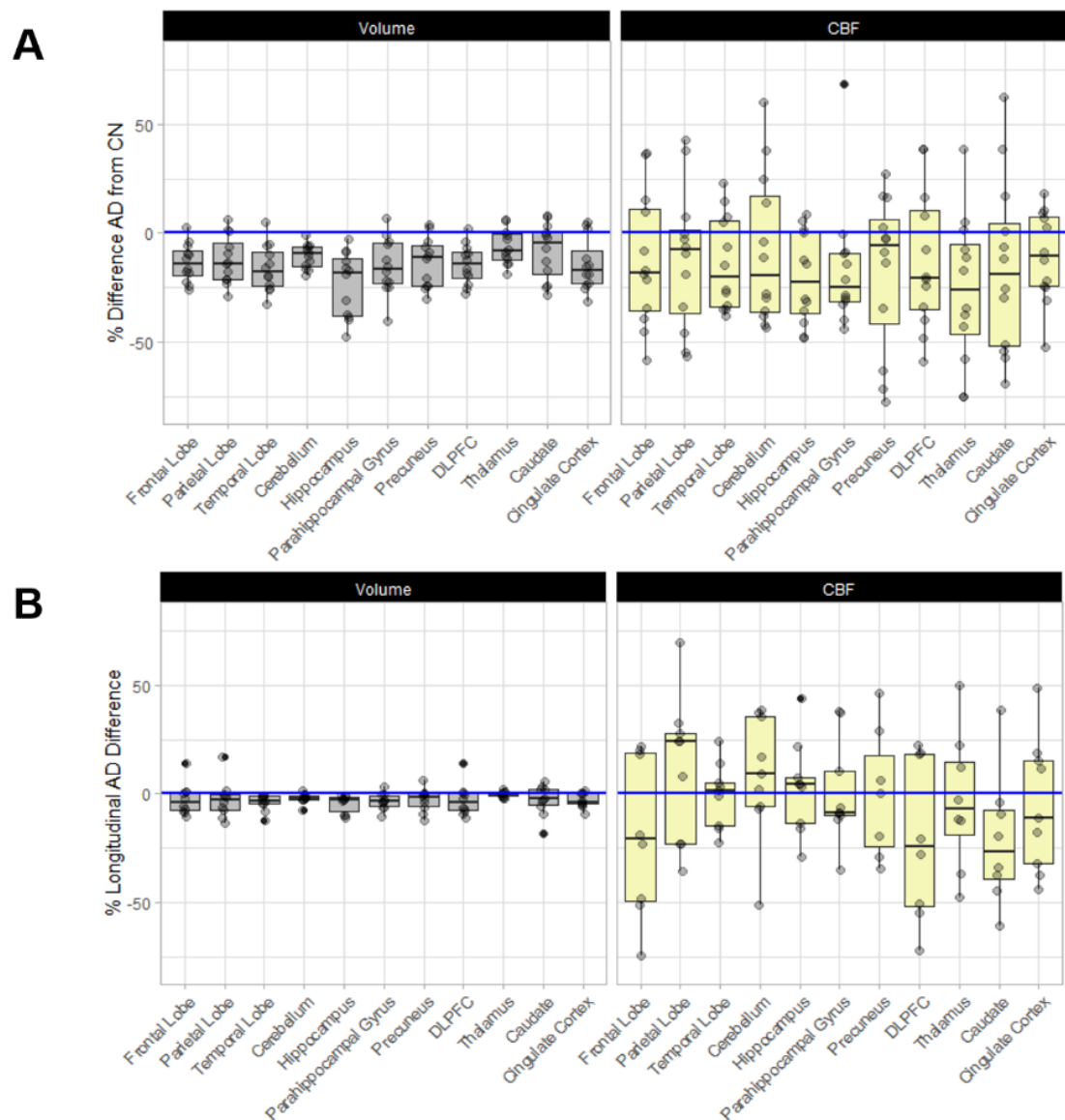
Measure	Baseline (n=8)		Longitudinal (n=8)		Δ %	p-value ¹
	Mean	SD	Mean	SD		
MMSE	24.38	3.96	23.75	5.20	-3	0.629
ACE Total	79.25	11.08	77.00	14.00	-3	0.372
Attention	15.25	2.76	14.25	4.40	-7	0.227
Memory	18.12	5.82	14.50	6.00	-25	0.005
Fluency	8.25	2.25	8.38	2.77	1	0.879
Language	23.00	2.27	24.50	1.60	6	0.072
Visuospatial	14.50	1.31	14.75	1.75	2	0.780
¹ Paired two-tailed t-test assuming unequal variance						

Brain atrophy and reduced brain perfusion in AD brains

At baseline, all ROIs had smaller volumes in the AD patients compared with controls. The greatest relative differences in volumes were in the hippocampus (-23%, $p < 0.001$) and temporal lobe (-16%, $p < 0.001$) (Figure 4.1A). Partial volume corrected data were used as the primary outcome parameters for the PET and CBF assessments. Uncorrected data was generally consistent with partial volume corrected data and is provided as Supplementary Information.

CBF was lower in AD patients compared to controls in all brain regions (range -8 to -26%). The greatest relative reductions in CBF were observed for the hippocampus (-20%, $p = 0.023$) and thalamus (-26%, $p = 0.044$) (see Supplementary Table S4.1 and Figure 4.1A).

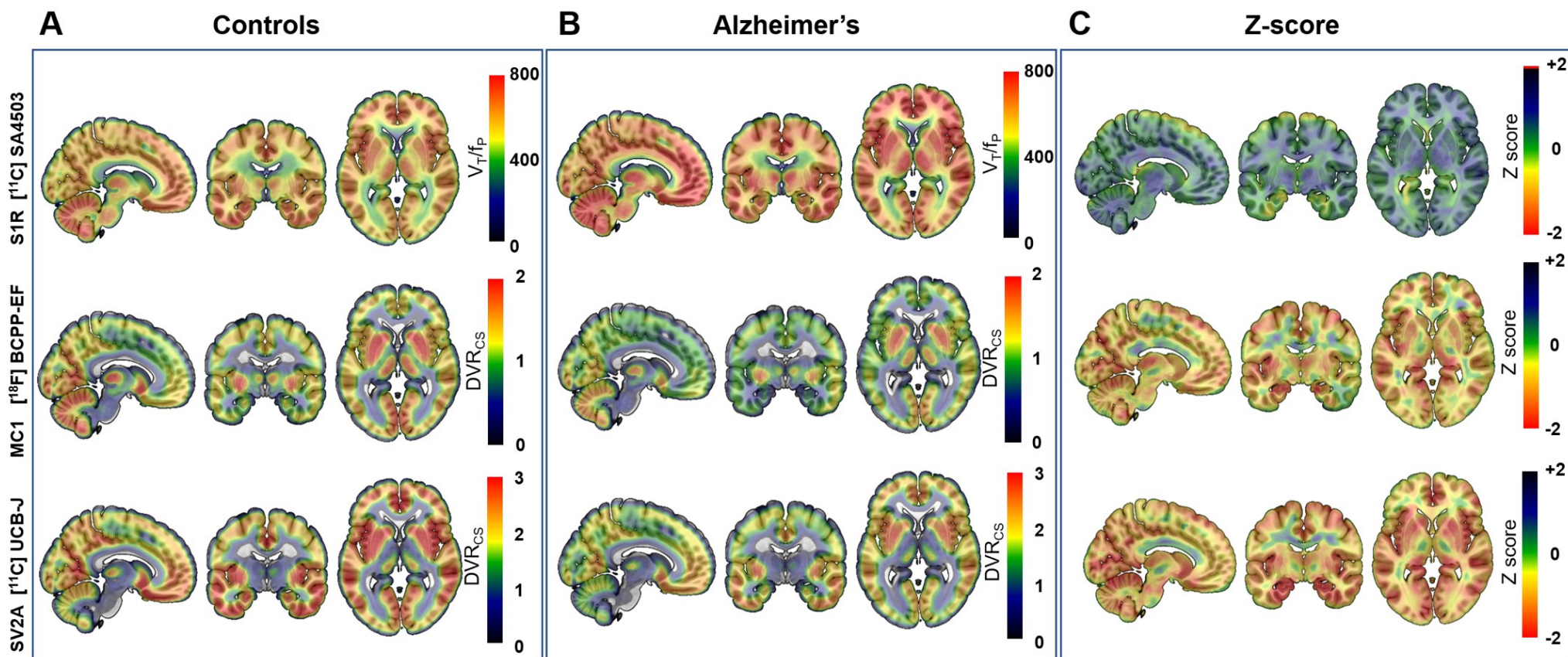
Figure 4.1. Cross-sectional and longitudinal results for volume and CBF. Figure showing cross-sectional results (A) and longitudinal results (B) for volume (left in grey) and CBF (right in yellow). Panel A shows box and whisker plots of individual AD patient percentage differences from the mean controls corresponding with values in Supplementary Table 1. Panel B shows box and whisker plots of longitudinal AD patient percentage change from baseline corresponding with values in Supplementary Table S4.2



Widespread increases in brain oxidative stress responses in AD brains

[¹¹C]SA4503 V_T/f_P was increased in all brain ROIs except the hippocampus and caudate in AD patients compared to controls (Figure 4.2, 4.3A and Supplementary Table S4.1; see also Supplementary Table 3 for radioligand binding measures without partial volume correction). The parametric image contrasting mean voxel-wise [¹¹C]SA4503 V_T/f_P for AD patients with the controls was consistent with ROI data (Figure 4.2) in defining the largest and most statistically significant relative increases in [¹¹C]SA4503 binding in the cingulate cortex (+27%, $p=0.010$), the precuneus (+23%, $p=0.011$), cerebellum (+24% $p=0.030$) and parietal (+21%, $p=0.023$) and temporal (+21%, $p=0.033$) lobes. No significant association was found between [¹¹C]SA4503 V_T/f_P and CBF in any of the ROIs for the AD patients or controls.

Figure 4.2. Mean voxel-wise PET images for controls, AD, and z-score contrasting AD from controls. Mean voxel-wise PET images in MNI 152 space for controls (n=16) (A), AD patients at baseline (n=12) (B) and the Z-difference image contrasting the AD patients with controls (C) for [^{11}C]SA4503 V_T/f_p (sigma 1 receptor) (top), [^{18}F]BCPP-EF DVR_{CS} (mitochondrial complex I) (middle) and [^{11}C]UCB-J DVR_{CS} (synaptic density) (bottom).



Focal decreases in synaptic density in AD brains

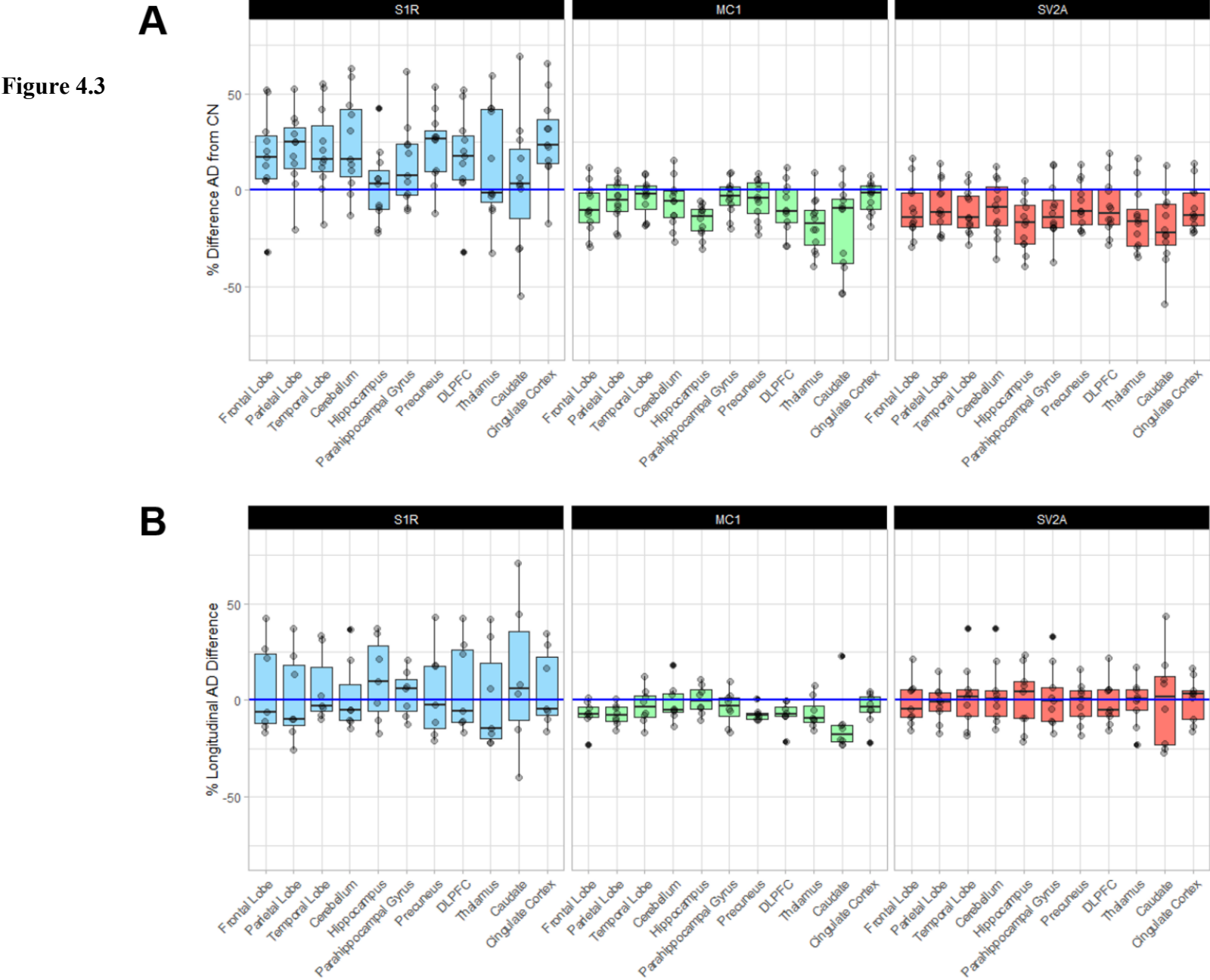
[¹¹C]UCB-J DVR_{CS} was generally lower in brains of the AD patients compared with controls, although not significantly reduced in all ROIs (Figure 4.2, 4.3A and Supplementary Table S4.1, see also Supplementary Table S4.3 for binding measures without volume correction). The greatest relative reductions in SV2A density were found in the caudate (-25%, $p=0.007$), hippocampus (-24%, $p=0.001$) and thalamus (-19%, $p=0.012$). No significant association was found between [¹¹C]UCB-J DVR_{CS} and CBF in any of the ROIs for the AD patients or for controls.

Focal decreases in mitochondrial complex I in AD brains

[¹⁸F]BCPP-EF DVR_{CS}, which provides a measure of the density of MC1, the first enzyme in the mitochondrial electron transport chain, was lower in the brains of AD patients compared with controls, although it was not significantly reduced in all ROIs (Figure 4.2, 4.3A and Supplementary Table S4.1, see also Supplementary Table S4.3 for binding measures without volume correction). The greatest differences in [¹⁸F]BCPP-EF DVR_{CS} for the AD patients relative to controls were found in the hippocampus (-25%, $p = 0.02$), thalamus (-23%, $p =0.001$) and caudate (-28%, $p =0.02$), with smaller relative reductions in the frontal lobe (-12%, $p =0.040$) and dorsal lateral prefrontal cortex (DLPFC) (-12%, $p =0.048$). The parametric images comparing [¹⁸F]BCPP-EF DVR_{CS} between the two groups (Figure 4.2) highlighted the

larger focal differences. I did not find correlations between regional CBF and reduced [^{18}F]BCPP-EF DVR_{CS} in ROIs in AD patients or controls.

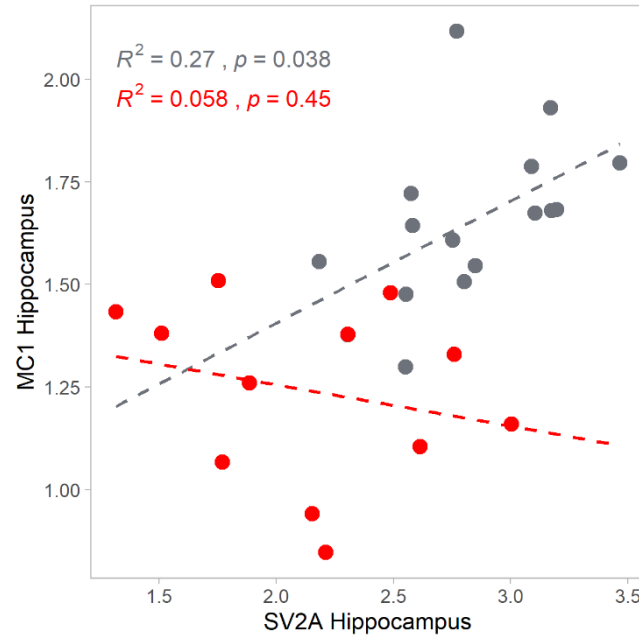
Figure 4.3. Cross-sectional differences between AD patients and controls, and longitudinal differences for AD patients between S1R, MC1, and SV2A. Cross-sectional differences between regional measures in AD patients (n=12) with the corresponding measures in mean controls (n=16) (A) and changes in individual AD patients between baseline and the 12-18 month follow up (B). Panel A shows box and whisker plots of individual Alzheimer's patient percentage differences from the mean control values [^{11}C]SA4503 V_T/f_p (sigma 1 receptor) (left in blue), [^{18}F]BCPP-EF DVR_{CS} (mitochondrial complex I) (middle in green) and [^{11}C]UCB-J DVR_{CS} (synaptic density) (right in red) (Supplementary Table S4.1). Panel B shows box and whisker plots of longitudinal AD patient percentage differences at 12-18 month follow up relative to baseline measures (Supplementary Table S4.2).



Mitochondrial oxidative phosphorylation is uncoupled from synaptic density in AD

MC1 catalyses a first, rate-limiting step in mitochondrial oxidative phosphorylation. [^{18}F]BCPP-EF DVR_{CS} and [^{11}C]UCB-J DVR_{CS} were positively correlated in controls in all regions, particularly frontal ($r^2=0.67$, $p=0.0001$), parietal ($r^2=0.66$, $p=0.00012$) and hippocampal ($r^2=0.27$, $p=0.038$) cortex, consistent with a physiological coupling between oxidative energy production and synapse density (see Figure 4.4).(151) However, this association was lost in the AD patients (e.g., in the hippocampus $r^2 = 0.058$, $p=0.45$).

Figure 4.4 Associations between MC1 and SV2A in the hippocampus for controls and AD patients. Associations between [^{18}F]BCPP-EF DVR_{CS} (MC1, ordinate) and [^{11}C]UCB-J DVR_C (SV2A, abscissa) in hippocampus for controls (grey) and AD patients (red) . [^{18}F]BCPP-EF DVR_{CS} and [^{11}C]UCB-J DVR_{CS} were moderately to strongly positively correlated in controls all regions, consistent with a physiological coupling between oxidative energy production and synapse density,(151) and this association was reduced or absent with Alzheimer's disease.

Figure 4.4

Regional longitudinal changes in metabolic and synaptic markers were consistent with differences between AD patients and controls at baseline

The between group comparisons at baseline suggested regional differences in the rates of progression of biochemical and synaptic pathology in AD. I re-scanned the AD group after 12-18 months. As expected, I found small global decreases in brain volume at follow up, with greater relative volume changes in the temporal lobe (-4%, $p=0.009$), hippocampus (-5%, $p=0.01$), parahippocampal gyrus (-3%, $p=0.027$), cingulate cortex (-3%, $p=0.023$) and cerebellum (-2%, $p=0.020$). There were no significant changes in CBF (Figure 4.1B, Supplementary Table S4.2).

Longitudinal PET measures of the biochemical pathology showed regional changes consistent with differences between the AD patients and controls at baseline (Figure 4.3B and Supplementary Table S4.2, also see Supplementary Table S4.4 for binding measures without volume correction). Regional [^{11}C]UCB-J DVR_{CS} was generally lower after 12-18 months, but the changes were small and none were statistically significant. [^{11}C]SA4503 $\text{V}_{\text{T}}/\text{f}_{\text{P}}$ showed non-significant trends for regional increases. By contrast, [^{18}F]BCPP-EF DVR_{CS} was decreased in all regions except the hippocampus and was most sensitive to change over time, with the largest relative reductions found in the parietal lobe (-11%, $p=0.009$), the precuneus (-10%, $p=0.001$), and the DLPFC (-12%, $p=0.037$).

Variable increases in [^{11}C]SA4503 $\text{V}_{\text{T}}/\text{f}_{\text{P}}$ with generally greater decreases in [^{18}F]BCPP-EF DVR_{CS} and [^{11}C]UCB-J DVR_{CS} in the prefrontal cortex were localised in the mean voxel-wise Z-images contrasting follow up with baseline for the AD patients (Figure 4.5).

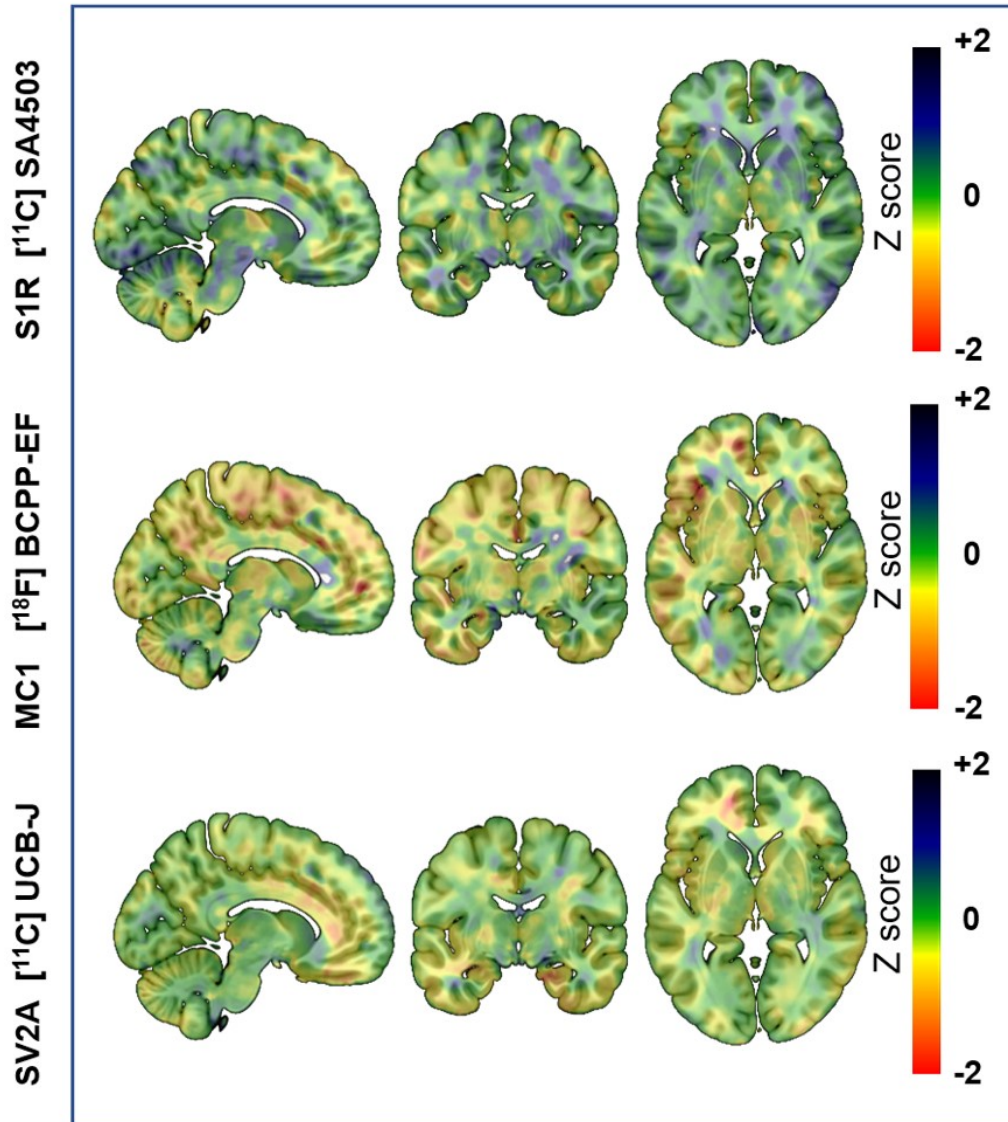


Figure 4.5 Voxel-wise Z score PET images of longitudinal change in AD patients.

Voxel-wise Z score PET images of longitudinal changes in AD patients, contrasting the 12-18 month follow up with baseline images in MNI 152 space for [^{11}C]SA4503 V_T/f_P (sigma 1 receptor) (top), [^{18}F]BCPP-EF DVR_{CS} (mitochondrial complex I) (middle) and [^{11}C]UCB-J DVR_{CS} (synaptic density) (bottom).

Loss of functional reserve in AD

I explored whether the AD patients had reduced brain functional reserve by exploring correlations between measures of mitochondrial oxidative capacity, synaptic density, and cell stress and measures of cognitive performance. [^{18}F]BCPP-EF DVR_{CS} showed negative associations with visuospatial scores in the AD patients ($r^2 = 0.45$, $p=0.016$), but not in controls ($r^2=0.076$, $p=0.3$) (Supplementary Figure S4.1). Hippocampal [^{11}C]SA4503 V_T/f_P showed significant negative associations with visuospatial scores in AD patients ($r^2 = 0.57$, $p=0.0076$) not found for the controls ($r^2 = 0.0061$, $p=0.77$). Associations of cognitive performance with synaptic density (Supplementary Figure S4.2) [^{11}C]UCB-J DVR_{CS} and language function tests (picture naming and semantic fluency) showed strong positive correlations in the AD patients (hippocampus, $r^2 = 0.68$, $p=0.0001$, parietal lobe, $r^2 = 0.39$, $p=0.03$), but not in controls (hippocampus, $r^2 = 0.0004$, $p=0.94$, parietal lobe, $r^2 = 0.009$, $p = 0.73$). Finally, I explored correlates of changes in memory over time in the AD patients (Supplementary Figure S4.3). Longitudinal decreases in frontal [^{18}F]BCPP-EF DVR_{CS} ($r^2 = 0.61$, $p=0.021$) were associated with decreases in ACE memory scores. Longitudinal increases in frontal lobe [^{11}C]SA4503 V_T/f_P were associated with decreases in ACE memory scores ($r^2 = 0.64$, $p=0.031$) .

Frontal, Parietal, and Temporal areas show marked relative differences across MC1, SV2A and S1R together in AD

In order to explore the relationship between MC1, SV2A and S1R in the cellular phase of AD a voxel-wise orthogonal regression by PCA was carried out by fitting a hyperplane to explain most variance in AD relative to controls. Outlier voxels $> 2SD$ from the mean perpendicular distance of an individual voxel to the hyperplane intersection of the control group were mapped to anatomy. My findings showed regionally localised relative differences across MC1, SV2A, and S1R in Alzheimer's in frontal, parietal and temporal cortices and subcortical areas (Figure S4.5) with the greatest z score being in parahippocampal gyrus at 4.3. Notably the AD areas with least standard deviation from CN were the occipital lobe and cerebellum.

Discussion

My study is unique for its *in vivo* multi-modal, longitudinal characterisation of molecular markers related to mitochondrial and synaptic dysfunction in early AD. I exploited recently introduced PET markers of cellular stress, mitochondrial and synaptic density to explore *in vivo* pathology in AD. Highly specific molecular markers were complemented by more generalised physiological perfusion (ASL) and volumetric MRI measures. Observation of increased S1R density in AD provides evidence of a generalised ER stress response. More focal decreases in synaptic and

mitochondrial density, seen particularly in the hippocampus, thalamus and caudate, suggest selective regional vulnerability to this generalised biochemical stress. The positive correlation between hippocampal mitochondrial and synaptic density found in controls was not seen in the AD patients, providing evidence for a loss of the normal physiological relationship between mitochondrial oxidative phosphorylation and synaptic density in disease. (151) Longitudinal follow-up of the AD patients showed that the measures of mitochondrial activity and brain volume loss were most sensitive to disease progression, although non-significant changes in other measures were broadly consistent with their relative differences between AD and controls at baseline. Exploratory analyses showed associations between regional mitochondrial, synaptic and sigma-1 receptor density (a marker of cellular stress) with impaired visuospatial function, language and memory test performance in the AD patients, but not in controls, suggesting loss of synaptic and metabolic functional reserve with disease. Overall, my results are consistent with a disease model in which generalised cell stress leads to regionally variably expressed pathologies of mitochondrial dysfunction, synaptic loss and clinical expression of AD.

The S1R is a pluripotent modulator of multiple systems (59) found at the ER-mitochondrial interface (MAM) and expressed widely throughout the CNS. (60) S1R is a chaperone protein that stabilises IP3R-VDAC interactions mediating Ca^{2+} flux ER stores to the mitochondria. Mitochondrial ATP production is highly Ca^{2+} dependent,

and S1R expression promotes cellular survival by regulating Ca^{2+} signalling from the ER into mitochondria and enhancing ER–nuclear signalling for antioxidant protection via the serine/threonine-protein kinase/ endoribonuclease inositol-requiring enzyme 1 α (IRE1) and nuclear factor erythroid 2-related factor 2 (Nrf2). (59) (55) (25). Increased expression of S1R provides an index of adaptive responses to cell stress and [^{11}C]SA4503 PET imaging has been validated as a measure of the distribution of S1R in the brain(103) (104) (105). Using [^{11}C]SA4503 PET imaging, I have provided evidence for the first time of widespread and generalised increases in S1R density in early AD patients. An earlier study reported that S1R binding was decreased in AD(106), but the investigators scanned participants who were being treated with donepezil, which confounds interpretation as it has high affinity for S1R(152) and thus blocks the binding of [^{11}C]SA4503 to the S1R. I hypothesise that the generalized increase in S1R binding in AD patients is a consequence of interactions between aging and genetic risk susceptibility that are expected to act throughout the brain. However, my results provide evidence that the expression of S1R also must be influenced by regionally specific factors: S1R binding was not increased in patients with AD in the hippocampus, the brain region that showed the biggest differences in mitochondrial and synaptic density compared to controls. This may reflect a failure of adaptive mechanisms for cell stress, significant degeneration of cells expressing S1R in hippocampus or – as the range of disease stage included in my study was limited - a non-linear (“inverted U”) response with progression of biochemical pathology affecting other regions at later stages of disease. Future longitudinal imaging studies

of people at high risk of AD prior to clinical expression or of those with early mild cognitive impairment and direct characterisations of oxidative and ER stress markers and S1R expression in related hippocampal tissue *post-mortem* could help to resolve this question.

MC1 is the first enzyme in the mitochondrial electron transport chain. (70) [^{18}F]BCPP-EF was developed as a PET radioligand to image MC1 density *in vivo* (107,110,111) and the first full human characterisation was completed only recently (105,112). I found decreases in MC1 binding in AD patients greater than previously reported(115,116), most prominently in the hippocampus and in the highly connected subcortical grey matter of the caudate and thalamus. [^{18}F]BCPP-EF DVR_{CS} was the most sensitive longitudinal marker of disease progression of those that I explored. While all brain regions showing relative changes at baseline also showed changes over time, the largest longitudinal decreases were found in brain regions associated with early amyloid deposition (the posterior cingulate cortex, the precuneus, and the medial orbitofrontal cortex). (34) The observation that CBF and MC1 binding were not correlated in AD patients argues that the impaired mitochondrial biogenesis or dysfunction is independent of perfusion deficits. (153)

Synaptic loss begins early and progresses in AD (28); loss or dystrophy of dendritic spines is associated with β -amyloid fibrils and oligomers in preclinical models of AD pathology. (154) Indirect measures of synaptic loss as changes in brain functional MRI network topology have previously been associated with the expression of major symptoms of the disease. (155) *In vivo* human imaging studies based on PET radioligand binding to the synaptic vesicle glycoprotein 2A (SV2A)(74,119) showing reduced tissue SV2A density highlight that relative cortical volume reductions (52) underestimate the extent of synaptic loss in AD. However, although I observed some volume loss over the short 12-18 month longitudinal follow up, I could not measure significant reductions of SV2A density in the remaining brain tissue. Future studies with larger numbers of participants or longer periods of follow up will be needed to estimate the rate of SV2A marker loss independent of the progressive brain atrophy in AD and to identify where and when it is most sensitive to disease progression.

As well as underestimating the magnitude of synaptic loss across the brain, its distribution also is not fully explained by the regional brain volume loss. I found large reductions in SV2A in AD patients' grey matter, particularly in the hippocampus, caudate and thalamus. As there is less relative atrophy of the subcortical nuclei in AD disease than in the hippocampus, I speculate that the high synaptic loss seen in these nuclei reflects loss of convergent projections from widespread cortical regions, in addition to any local neurodegeneration. This observation further emphasises that

consideration both of synaptic losses due to atrophy and the reduced density in the remaining tissue due to connectivity disruption are needed to provide a full index of synaptic loss in AD. This can be appreciated quantitatively from comparison of partial volume corrected (Supplementary Table S4.1) regional SV2A measures and those without this correction, which show greater and more significant differences between AD patients and controls (Supplementary Table S4.3).

Cognitively normal controls did not show meaningful associations of cognitive performance measures with levels of S1R, MC1 or SV2A, consistent with functional reserve in the healthy older brain. Visuospatial, language and memory performance were correlated with hippocampal and frontal lobe S1R, MC1 or SV2A for AD patients. This suggests that functional reserve present in the healthy brain is lost in early AD, in which mitochondrial activity, oxidative stress responses and synaptic density appear to become rate limiting for cognition. If these correlations can be shown to reflect causal relations, therapeutic targeting of the responsible mechanisms could provide clinical benefits and be monitored using the measures here.

The relatively small size of the group of AD patients and the short period of follow up are obvious limitations to my study. Reflecting the broadly exploratory nature of the investigation, I did not correct statistical interrogations for multiple comparisons, so

the observations may include false positive (and negative) signals. Both of these factors may have influenced the apparent extent of differences seen between AD patients and controls. Against this, the qualitative differences in unthresholded Z-difference maps are consistent with my major conclusions from the ROI analysis that there are widespread differences in S1R and more focal differences in MC1 and SV2A between AD and controls. I have expressed all of my PET results after partial volume correction given the significant volumetric differences seen in my data and the confound of partial volume effects on the interpretation of tissue density measures. Partial volume correction algorithms are not perfect and introduce additional variability into the data. However, measures without partial volume correction (Supplementary Tables S4.3 and S4.4) are consistent with my conclusions as the relative magnitude of volume loss is lower than that of MC1 or SV2A PET signal loss. My healthy control and AD patient groups, although matched for sex, had a mean age difference of about 10 years. Data from my full cohort of MIND MAPS healthy volunteers across larger age range (20-80 years old) allowed us to estimate the expected age-related changes in PET parameters to be approximately +0.05 to +0.1%/year for S1R binding (n=24), -0.2 to -0.7%/year for MC1 binding (n=31), and -0.02 to -0.35%/year for SV2A binding (n=24) across frontal, parietal, hippocampus and caudate ROIs. As the differences seen between the AD patients and healthy controls reported here are substantially greater than these, I do not believe that the age difference between groups is relevant to the interpretation of my results. This cohort of patients is less representative of the AD patients taking donepezil. However, given a 1/3 of dementia is undiagnosed in the UK

(and are therefore not on symptomatic therapy), and only 1/2 of those with mild dementia are taking symptomatic therapies which may include either donepezil, galantamine and rivastigmine, our cohort is generally representative for this early stage of disease. Our amyloid positive cohort is likely an earlier cohort with 6 MCI, and 6 early AD together making up the group. Further studies are needed to look at the effects of medications on these molecular effectors. Finally, there are obvious limitations when using single molecular markers for each type of pathology to define more general pathological concepts. For example, recent data raises the possibility that SV2A density may reflect GABAergic inhibitory synapses preferentially over glutamatergic synapses. (156) However, MC1 density provides a more specific index of capacity for mitochondrial respiratory oxidative phosphorylation than does the alternative [¹⁸F]2-Fluoro-2-deoxy-D-glucose PET measure, which primarily reflects hexokinase activity, and thus can be confounded by differences in glycolytic activity due to disease and inflammation). Finally, although S1R is a non-specific marker of cell stress, the information it provides as a non-invasive, *in vivo* measure is unique and, in the context of AD, can be interpreted in terms of oxidative and ER stress with some confidence. (157,158)

My study illustrates the potential value of multi-modal, longitudinal imaging studies in AD, where no single measure describes a sufficient breadth of pathology to fully characterise disease progression. Ours is the first such study to date to include

longitudinal observations which support and extend cross-sectional ones. The results emphasise that, despite the magnitude of regional changes (e.g., in the hippocampus), cell stress responses in AD are generalised, suggesting a widespread, prodromal biochemical pathology. Elucidating mechanisms linking genetic susceptibility to this biochemical pathology will be important for prioritising targets for earliest interventions to delay or reverse disease.

One of the major findings in this work was increased S1R density in AD compared to controls, interpreted as widespread cell stress in the context of mitochondrial dysfunction. Given the large number of genes involved in lipid handling in AD and S1R association with lipid handling it follows that S1R might be modulated by lipid loading. This could be further investigated using transcriptomic data to give further insight into the behaviour of S1R. Using PET, an experimental medicine paradigm could be used by characterising the S1R pre and post HMG-CoA reductase inhibitor (statin) in an early population to see if lipid handling was specifically involved in this process *in vivo*.

Supplementary Materials

Table S4.1. Baseline [^{11}C]SA4503 V_T/f_p (sigma 1 receptor), [^{18}F]BCPP-EF DVR_{CS} (mitochondrial complex I), [^{11}C]UCB-J DVR_{CS} (synaptic density), MRI ASL cerebral blood flow (CBF) (all partial volume corrected) and normalised brain volumes (volume) measures in AD patients (n=12) and controls (n=16).

ROI	[^{11}C]SA4503 V_T/f_p – volume corrected							[^{18}F]BCPP-EF DVR_{CS} – volume corrected							[^{11}C]UCB-J DVR_{CS} – volume corrected							Volume (g)							CBF (ml/100g/min) – volume corrected						
	AD mean	sd	CN mean	sd	% AD from CN	p-value ¹	adjusted p-value ²	AD mean	sd	CN mean	sd	% AD from CN	p-value ¹	adjusted p-value ²	AD mean	sd	CN mean	sd	% AD from CN	p-value ¹	adjusted p-value ²	AD mean	sd	CN mean	sd	% AD from CN	p-value ¹	adjusted p-value ²	AD mean	sd	CN mean	sd	% AD from CN	p-value ¹	adjusted p-value ²
Frontal Lobe	1537	304	1306	366	18	0.088	0.140	2.93	0.55	3.33	0.39	–12	0.040	0.106	5.13	0.98	5.82	0.73	–12	0.052	0.082	699	71	809	86	–14	0.001	0.002	40.05	14.14	45.56	8.96	–12	0.256	0.313
Parietal Lobe	1428	230	1183	295	21	0.023	0.072	3.12	0.46	3.36	0.40	–7	0.165	0.245	5.11	0.87	5.65	0.67	–10	0.089	0.092	810	102	923	84	–12	0.005	0.006	39.15	14.43	44.31	9.99	–12	0.306	0.336
Temporal Lobe	1403	257	1162	292	21	0.033	0.072	2.55	0.35	2.69	0.29	–5	0.273	0.273	4.65	0.73	5.34	0.49	–13	0.010	0.036	991	128	1181	113	–16	0.000	0.002	37.55	9.54	43.60	7.86	–14	0.091	0.313
Cerebellum	1415	285	1142	329	24	0.030	0.072	2.60	0.47	2.87	0.25	–9	0.098	0.180	2.81	0.61	3.19	0.31	–12	0.061	0.084	860	54	961	66	–11	0.000	0.001	29.82	11.24	32.38	9.12	–8	0.529	0.529
Hippocampus	949	176	929	282	2	0.821	0.821	1.24	0.22	1.65	0.20	–25	0.000	0.000	2.15	0.52	2.82	0.36	–24	0.001	0.012	58	11	75	6	–23	0.000	0.001	42.21	11.23	53.04	11.87	–20	0.023	0.242
Parahippocampal Gyrus	1222	231	1077	253	13	0.137	0.188	1.86	0.27	1.96	0.20	–5	0.271	0.273	3.42	0.72	3.96	0.36	–14	0.030	0.067	75	12	88	7	–15	0.002	0.004	42.62	15.03	50.70	11.88	–16	0.143	0.313
Precuneus	1389	212	1133	274	23	0.011	0.063	3.05	0.44	3.26	0.41	–7	0.210	0.257	4.89	0.75	5.40	0.67	–9	0.077	0.092	197	26	227	23	–13	0.004	0.006	36.19	15.80	43.91	12.69	–18	0.184	0.313
DLPFC	1508	297	1284	358	17	0.089	0.140	2.90	0.55	3.29	0.37	–12	0.048	0.106	5.12	0.97	5.71	0.71	–10	0.092	0.092	600	64	700	78	–14	0.001	0.002	40.39	14.90	46.24	9.66	–13	0.255	0.313
Thalamus	1246	320	1102	285	13	0.244	0.299	2.58	0.60	3.35	0.40	–23	0.001	0.007	2.96	0.76	3.66	0.53	–19	0.013	0.036	193	17	206	15	–6	0.043	0.047	31.31	14.51	42.38	11.72	–26	0.044	0.242
Caudate	692	225	668	182	4	0.770	0.821	1.63	0.73	2.28	0.57	–28	0.020	0.073	2.76	0.88	3.68	0.66	–25	0.007	0.036	43	6	47	6	–8	0.114	0.114	20.15	9.67	23.85	3.35	–16	0.228	0.313
Cingulate Cortex	1623	288	1280	345	27	0.010	0.063	2.83	0.32	2.99	0.25	–5	0.178	0.245	5.15	0.82	5.76	0.56	–11	0.039	0.071	331	47	386	43	–14	0.004	0.006	47.00	11.04	52.56	8.87	–11	0.171	0.313

¹Unpaired two-tailed t-test assuming unequal variance

²False Discovery Rate

Table S4.2. Mean [^{11}C]SA4503 V_T/f_p (sigma 1 receptor), [^{18}F]BCPP-EF DVR_{CS} (mitochondrial complex I), [^{11}C]UCB-J DVR_{CS} (SV2A synaptic density), MRI ASL cerebral blood flow (CBF) (all partial volume corrected) and normalised brain volumes (volume) measures in AD patients after 12-18 month follow up.

ROI	^[11C] SA4503 V _T /f _p – volume corrected							^[18F] BCPP-EF DVR _{CS} – volume corrected							^[11C] UCB-J DVR _{CS} – volume corrected							Volume (g)							CBF (ml/100g/min) – volume corrected						
	Baseline mean	sd	Longitudinal mean	sd	% from baseline	p-value ¹	adjusted p-value ²	Baseline mean	sd	Longitudinal mean	sd	% from baseline	p-value ¹	adjusted p-value ²	Baseline mean	sd	Longitudinal mean	sd	% from baseline	p-value ¹	adjusted p-value ²	Baseline mean	sd	Longitudinal mean	sd	% from baseline	p-value ¹	adjusted p-value ²	Baseline mean	sd	Longitudinal mean	sd	% from baseline	p-value ¹	adjusted p-value ²
Frontal Lobe	1435	270	1504	387	5	0.616	0.968	2.87	0.60	2.57	0.50	−12	0.052	0.121	5.00	1.00	4.93	1.25	−1	0.768	1.000	709	74	688	65	−3	0.208	0.257	40.97	15.24	36.77	22.33	−8	0.442	0.817
Parietal Lobe	1372	220	1373	282	0	0.991	0.991	3.09	0.54	2.78	0.51	−11	0.009	0.052	5.13	0.89	5.02	1.07	−2	0.587	1.000	816	108	789	82	−3	0.224	0.257	40.12	15.92	43.19	18.87	12	0.520	0.817
Temporal Lobe	1318	206	1375	152	4	0.512	0.968	2.41	0.33	2.30	0.30	−5	0.337	0.412	4.54	0.74	4.60	0.84	1	0.855	1.000	1003	122	964	127	−4	0.009	0.056	37.30	10.23	37.13	12.21	−1	0.928	0.928
Cerebellum	1296	205	1293	146	−0	0.981	0.991	2.49	0.46	2.42	0.35	−3	0.594	0.654	2.79	0.63	2.84	0.46	2	0.770	1.000	875	46	857	49	−2	0.020	0.060	31.94	11.91	34.76	17.99	8	0.500	0.817
Hippocampus	920	153	995	122	8	0.322	0.968	1.19	0.24	1.19	0.23	0	0.995	0.995	2.13	0.43	2.13	0.51	−0	0.996	1.000	58	11	55	11	−5	0.010	0.056	41.34	12.05	42.17	14.49	3	0.771	0.928
Parahippocampal Gyrus	1169	149	1201	113	3	0.572	0.968	1.79	0.29	1.67	0.24	−7	0.238	0.328	3.28	0.72	3.30	0.65	1	0.913	1.000	76	9	74	11	−3	0.027	0.060	42.99	17.10	42.44	15.84	1	0.853	0.928
Precuneus	1331	181	1361	263	2	0.807	0.986	3.04	0.50	2.75	0.53	−10	0.001	0.009	4.91	0.78	4.84	1.02	−1	0.757	1.000	200	25	194	24	−3	0.105	0.192	37.23	16.84	41.49	18.28	34	0.402	0.817
DLPFC	1412	268	1494	386	5	0.555	0.968	2.85	0.62	2.55	0.51	−12	0.037	0.121	5.01	1.01	4.94	1.24	−1	0.770	1.000	608	66	588	59	−3	0.187	0.257	41.60	16.00	37.07	23.23	−9	0.414	0.817
Thalamus	1200	294	1163	238	−3	0.756	0.986	2.52	0.67	2.28	0.62	−11	0.059	0.121	2.99	0.81	2.95	1.02	−1	0.841	1.000	193	14	193	14	−0	0.623	0.623	32.91	16.10	34.23	22.21	16	0.798	0.928
Caudate	669	172	729	329	8	0.616	0.968	1.69	0.51	1.35	0.66	−25	0.066	0.121	2.67	1.00	2.83	1.06	6	0.652	1.000	43	5	42	5	−3	0.233	0.257	20.58	10.46	18.38	13.56	−9	0.413	0.817
Cingulate Cortex	1518	237	1588	272	4	0.552	0.968	2.76	0.34	2.58	0.32	−7	0.203	0.318	5.03	0.83	5.03	0.94	0	1.000	1.000	337	49	327	52	−3	0.023	0.060	46.24	11.53	43.18	16.76	−5	0.477	0.817

¹Paired two-tailed t-test assuming equal variance

²False Discovery Rate

Table S4.3. Mean baseline [^{11}C]SA4503 V_T/f_p (sigma 1 receptor), [^{18}F]BCPP-EF DVR_{CS} (mitochondrial complex I), [^{11}C]UCB-J DVR_{CS} (synaptic density) (*without* partial volume correction) and normalised brain volumes (volume) measures in Alzheimer's patients (n=12) and controls (n=16).

Cross-sectional results of S1R, MC1 & SV2a in AD and CN controls																												
ROI	^[11C] SA4503 V _T /f _p – uncorrected							^[18F] BCPP-EF DVR _{CS} – uncorrected							^[11C] UCB-J DVR _{CS} – uncorrected							Volume (g)						
	AD mean	sd	CN mean	sd	% AD from CN	p-value ¹	adjusted p-value ²	AD mean	sd	CN mean	sd	% AD from CN	p-value ¹	adjusted p-value ²	AD mean	sd	CN mean	sd	% AD from CN	p-value ¹	adjusted p-value ²	AD mean	sd	CN mean	sd	% AD from CN	p-value ¹	adjusted p-value ²
Frontal Lobe	748	157	666	188	12	0.228	0.407	0.82	0.18	1.10	0.14	−26	0.000	0.000	1.71	0.32	2.16	0.29	−21	0.001	0.001	699	71	809	86	−14	0.001	0.002
Parietal Lobe	713	129	631	156	13	0.153	0.407	0.90	0.17	1.17	0.17	−23	0.000	0.000	1.72	0.35	2.18	0.30	−21	0.002	0.002	810	102	923	84	−12	0.005	0.006
Temporal Lobe	760	140	690	170	10	0.259	0.407	0.84	0.16	1.09	0.17	−22	0.001	0.001	1.88	0.34	2.43	0.25	−23	0.000	0.000	991	128	1181	113	−16	0.000	0.002
Cerebellum	922	180	787	218	17	0.092	0.407	1.33	0.27	1.59	0.18	−16	0.009	0.009	1.46	0.34	1.88	0.19	−22	0.002	0.002	860	54	961	66	−11	0.000	0.001
Hippocampus	652	140	635	157	3	0.781	0.824	0.44	0.21	0.83	0.17	−47	0.000	0.000	0.95	0.29	1.58	0.24	−40	0.000	0.000	58	11	75	6	−23	0.000	0.001
Parahippocampal Gyrus	682	121	670	162	2	0.824	0.824	0.55	0.15	0.79	0.14	−30	0.000	0.000	1.41	0.35	1.98	0.17	−29	0.000	0.000	75	12	88	7	−15	0.002	0.004
Precuneus	762	134	678	164	12	0.158	0.407	1.09	0.20	1.41	0.18	−22	0.000	0.000	1.99	0.41	2.49	0.33	−20	0.003	0.003	197	26	227	23	−13	0.004	0.006
DLPFC	749	157	668	187	12	0.233	0.407	0.84	0.18	1.12	0.14	−25	0.000	0.000	1.71	0.32	2.17	0.29	−21	0.001	0.001	600	64	700	78	−14	0.001	0.002
Thalamus	600	153	552	152	9	0.428	0.589	0.55	0.22	0.91	0.24	−40	0.000	0.000	0.61	0.21	0.98	0.24	−38	0.000	0.000	193	17	206	15	−6	0.043	0.047
Caudate	484	146	458	133	6	0.638	0.780	0.55	0.34	0.91	0.33	−40	0.009	0.009	0.95	0.42	1.53	0.37	−38	0.001	0.001	43	6	47	6	−8	0.114	0.114
Cingulate Cortex	868	170	750	204	16	0.116	0.407	0.97	0.11	1.25	0.15	−22	0.000	0.000	2.04	0.33	2.61	0.28	−22	0.000	0.000	331	47	386	43	−14	0.004	0.006

¹ Unpaired two-tailed t-test assuming unequal variance

² False Discovery Rate

Table S4.4. Mean [^{11}C]SA4503 V_T/f_p (sigma 1 receptor), [^{18}F]BCPP-EF DVR_{CS} (mitochondrial complex I), [^{11}C]UCB-J DVR_{CS} (synaptic density), MRI ASL cerebral blood flow (CBF) (*without* partial volume correction) and normalised brain volumes (volume) measures in Alzheimer’s patients after 12-18 month follow up.

ROI	^[11C] SA4503 V _T /f _p – uncorrected							^[18F] BCPP-EF DVR _{CS} – uncorrected							^[11C] UCB-J DVR _{CS} – uncorrected							Volume (g)						
	Baseline mean	sd	Longitudinal mean	sd	% from baseline	p-value [/]	adjusted p-value ²	Baseline mean	sd	Longitudinal mean	sd	% from baseline	p-value [/]	adjusted p-value ²	Baseline mean	sd	Longitudinal mean	sd	% from baseline	p-value [/]	adjusted p-value ²	Baseline mean	sd	Longitudinal mean	sd	% from baseline	p-value [/]	adjusted p-value ²
Frontal Lobe	698	128	710	101	2	0.776	0.991	0.79	0.15	0.65	0.20	−22	0.065	0.120	1.65	0.33	1.56	0.42	−6	0.451	0.779	709	74	688	65	−3	0.208	0.257
Parietal Lobe	680	117	671	109	−1	0.837	0.991	0.86	0.16	0.71	0.18	−21	0.008	0.046	1.71	0.35	1.57	0.33	−9	0.174	0.779	816	108	789	82	−3	0.224	0.257
Temporal Lobe	723	133	736	93	2	0.730	0.991	0.78	0.15	0.70	0.21	−11	0.169	0.232	1.85	0.34	1.78	0.26	−4	0.551	0.779	1003	122	964	127	−4	0.009	0.056
Cerebellum	860	136	861	94	0	0.991	0.991	1.27	0.24	1.20	0.21	−6	0.393	0.393	1.46	0.36	1.46	0.22	−0	0.992	0.992	875	46	857	49	−2	0.020	0.060
Hippocampus	645	162	619	145	−4	0.565	0.991	0.40	0.25	0.36	0.26	−10	0.278	0.306	0.93	0.21	0.88	0.19	−6	0.567	0.779	58	11	55	11	−5	0.010	0.056
Parahippocampal Gyrus	658	118	672	83	2	0.637	0.991	0.51	0.14	0.45	0.21	−15	0.195	0.239	1.35	0.31	1.31	0.22	−3	0.664	0.812	76	9	74	11	−3	0.027	0.060
Precuneus	731	128	733	127	0	0.960	0.991	1.08	0.22	0.92	0.22	−18	0.002	0.026	2.00	0.42	1.86	0.36	−7	0.197	0.779	200	25	194	24	−3	0.105	0.192
DLPFC	699	128	715	102	2	0.704	0.991	0.81	0.16	0.67	0.20	−22	0.053	0.117	1.66	0.34	1.57	0.42	−6	0.434	0.779	608	66	588	59	−3	0.187	0.257
Thalamus	569	135	556	108	−2	0.786	0.991	0.50	0.24	0.40	0.23	−27	0.040	0.117	0.60	0.21	0.54	0.22	−12	0.325	0.779	193	14	193	14	−0	0.623	0.623
Caudate	463	124	486	135	5	0.537	0.991	0.57	0.27	0.42	0.32	−36	0.046	0.117	0.91	0.49	0.92	0.43	2	0.897	0.987	43	5	42	5	−3	0.233	0.257
Cingulate Cortex	819	149	827	111	1	0.846	0.991	0.94	0.12	0.84	0.21	−12	0.097	0.153	2.01	0.34	1.92	0.31	−5	0.432	0.779	337	49	327	52	−3	0.023	0.060

¹ Paired two-tailed t-test assuming equal variance

² False Discovery Rate

Figure S4.1: Association between hippocampal [^{18}F]BCPP-EF DVR_{CS} (MC1, ordinate) and visuospatial scores (A) and hippocampal [^{11}C]SA4503 V_T/f_P (S1R, ordinate) and visuospatial scores (B) for controls (grey) and AD patients (red). Hippocampal [^{18}F]BCPP-EF DVR_{CS} were negatively associated with visuospatial scores for the AD patients, but not for controls. Hippocampal [^{11}C]SA4503 V_T/f_P were negatively associated with visuospatial scores for the AD patients, but not for controls.

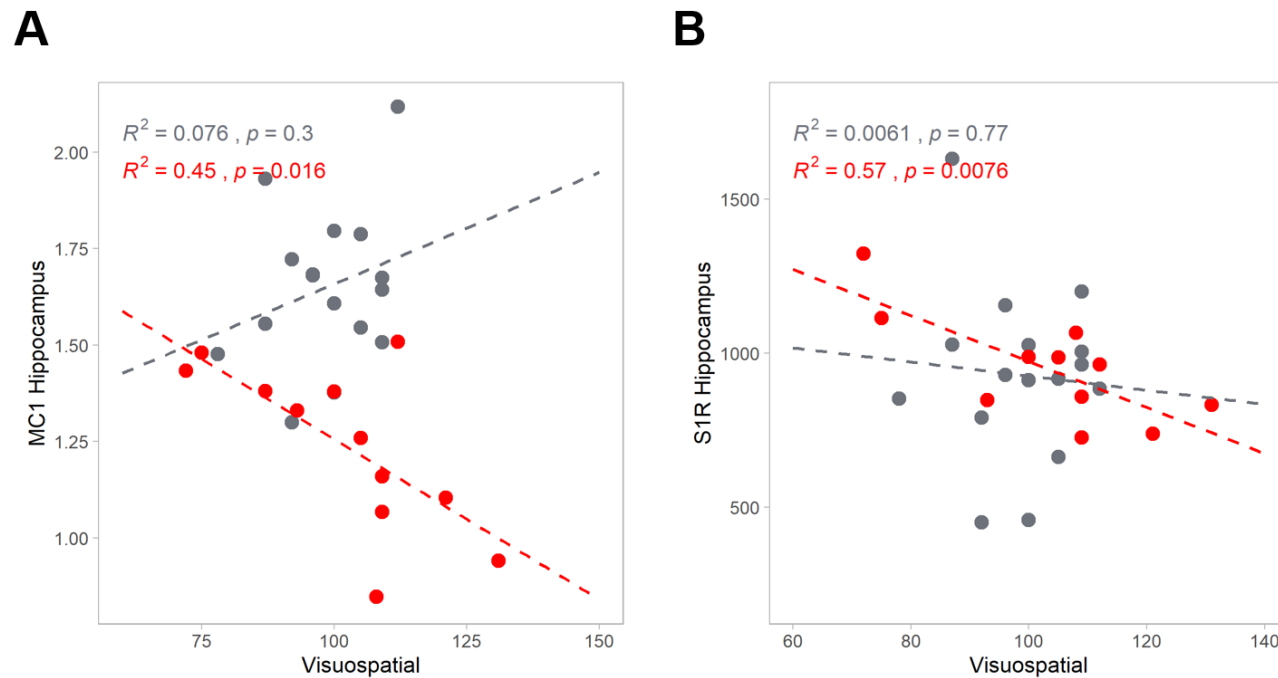


Figure S4.2:

Associations between hippocampal [^{11}C]UCB-J DVR_{CS} (SV2A synaptic density, ordinate) in the hippocampus (A) and in the parietal lobe [^{18}F]BCPP-EF DVR_{CS} (MC1, ordinate) and language scores for controls (grey) and AD (red) patients (A). Hippocampal [^{11}C]UCB-J DVR_{CS} binding and language function tests showed strong positive correlations for the AD patients, consistent with a loss of synaptic functional reserve, but not in controls.

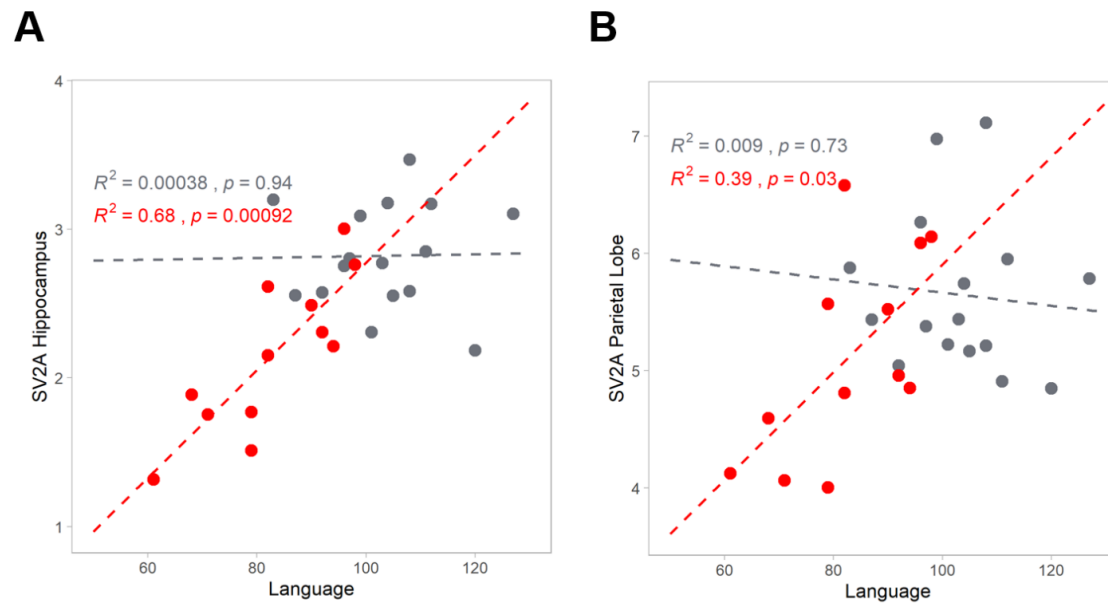


Figure S4.3:

Longitudinal percent decreases in frontal lobe [^{18}F]BCPP-EF DVR_{CS} (MC1) between baseline and 12-18 month follow up of the AD patients were associated with decreases in their ACE memory scores (B). Longitudinal percent decreases in frontal lobe [^{11}C]SA4503 V_T/f_P (sigma 1 receptor) (S1R) between baseline and 12-18 month follow up of the AD patients also were *negatively* associated with decreases in their ACE memory scores (B).

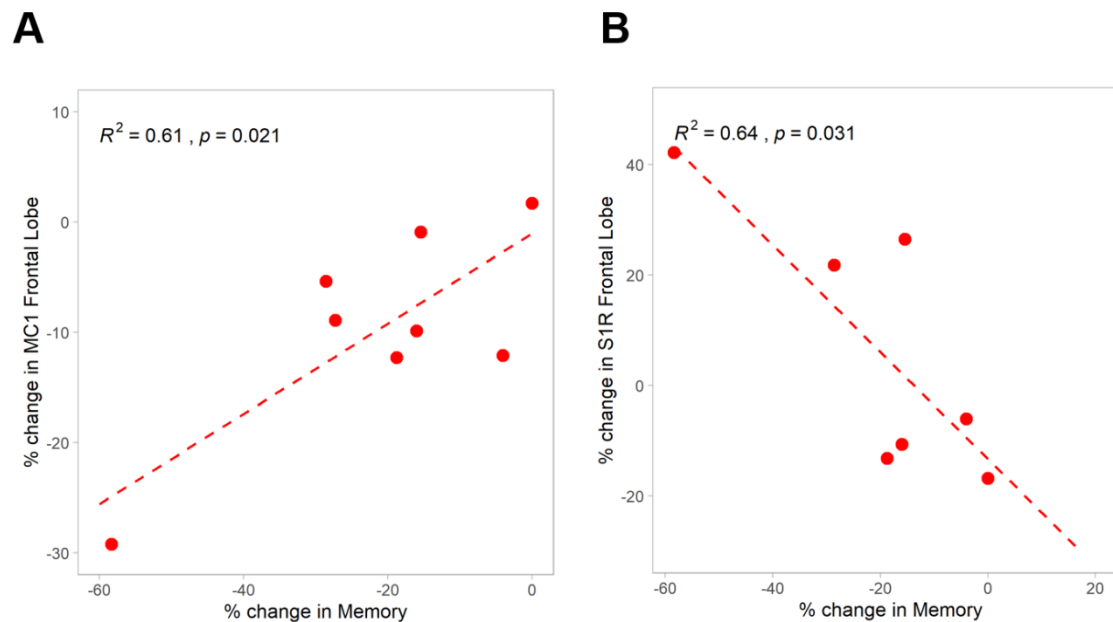


Figure S4.4: Pearson's r correlation matrix showing correlations between regional mean [^{11}C]SA4503 V_T/f_p (sigma 1 receptor), [^{18}F]BCPP-EF DVR_{CS} (mitochondrial complex I), [^{11}C]UCB-J DVR_{CS} (synaptic density) and MRI ASL cerebral blood flow (CBF) (all volume corrected) and clinical variables for controls (n=16) (A) and AD (n=12) patients (B) and for longitudinal changes in these for the AD patients (C). Scale for correlation values is shown on the right.

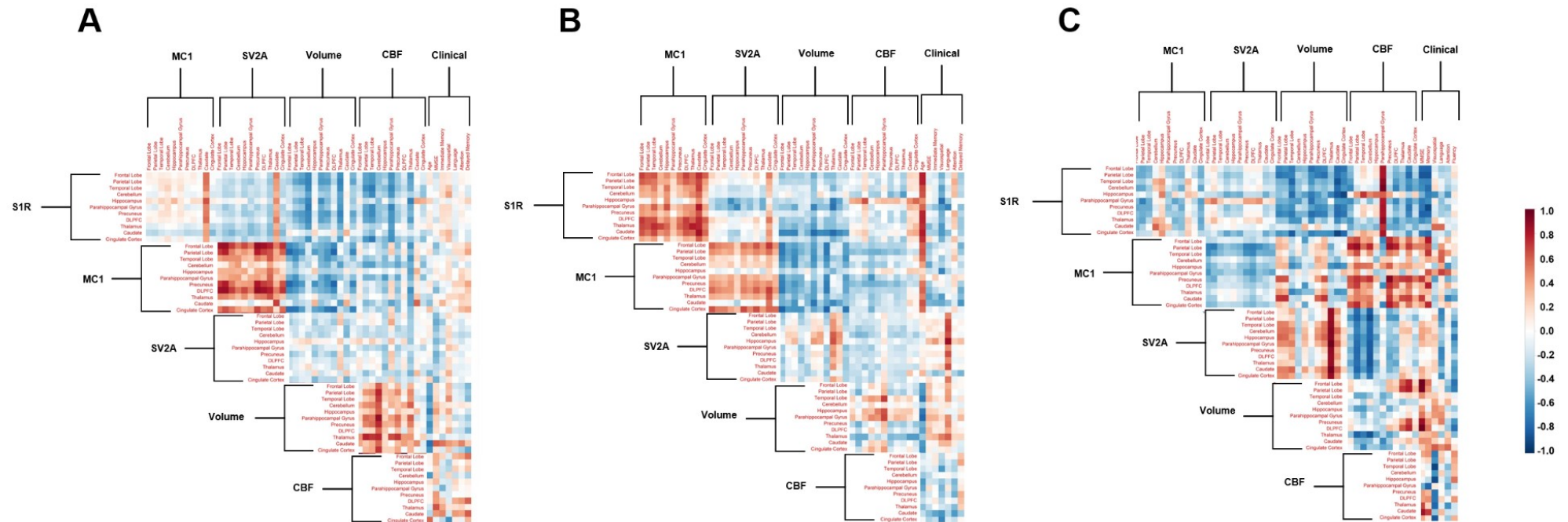


Figure S4.5

Figure showing MC1, SV2A and S1R voxels with a hyperplane fitted to the data for CN (panel A) and CN hyperplane fitted to AD (panel B). Outlier voxels $> 2SD$ from mean perpendicular distance of an individual voxel to the hyperplane intersection are labelled in red (CN outliers) and pink (AD outliers relative to CN) and shown for different 2D tracer views. Panel C shows AD z image of voxel-wise orthogonal regression by PCA mapped to anatomy (-2 black to +4 red) relative to fitted plane for CN voxels in coronal, axial, sagittal and rendered brain. These show regionally localised relative differences across MC1, SV2A, and S1R in Alzheimer's in frontal, parietal and temporal cortices and subcortical areas including anterior cingulate, dorsolateral frontal cortex, precuneous, medial frontal cortex, medial orbital cortex, parietal operculum, anterior temporal pole, temporal fusiform cortex, operculum, hippocampus, parahippocampal gyrus, superior and inferior temporal gyrus and pons.

Figure S4.5

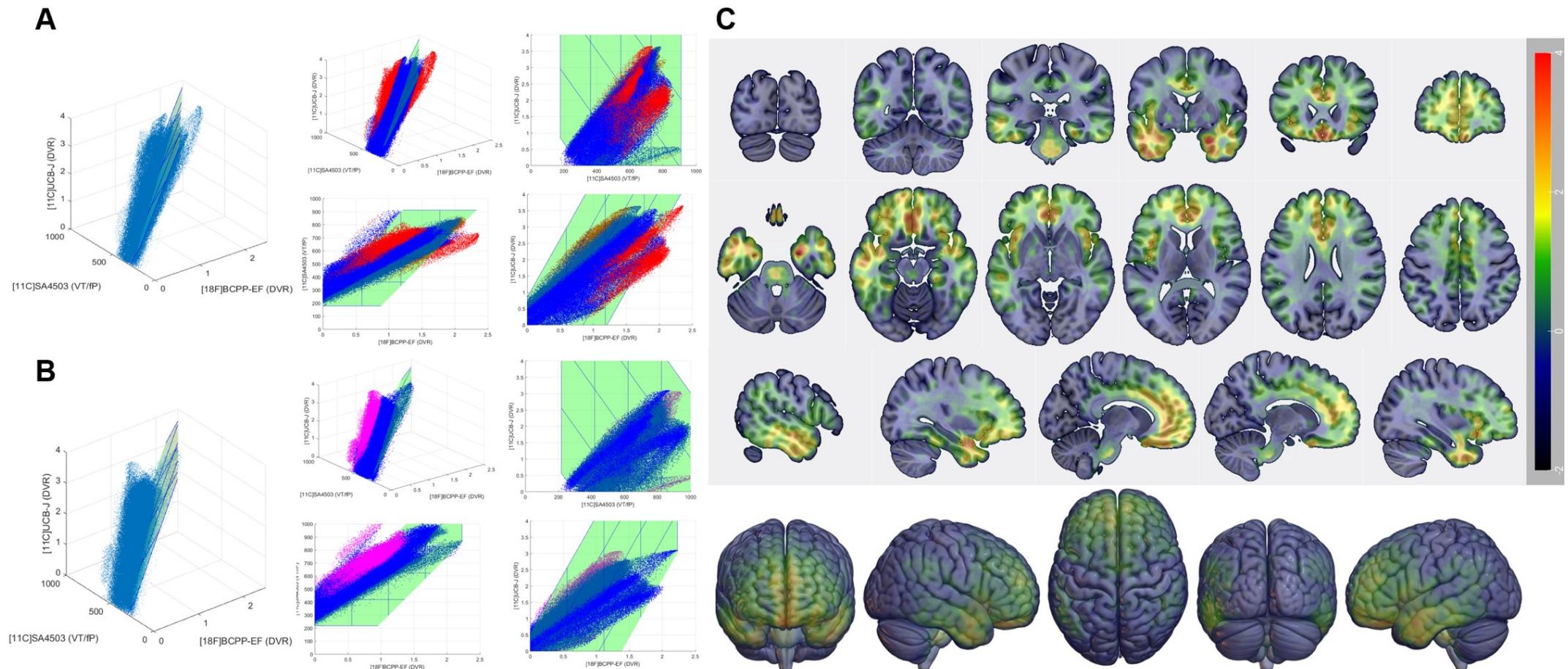
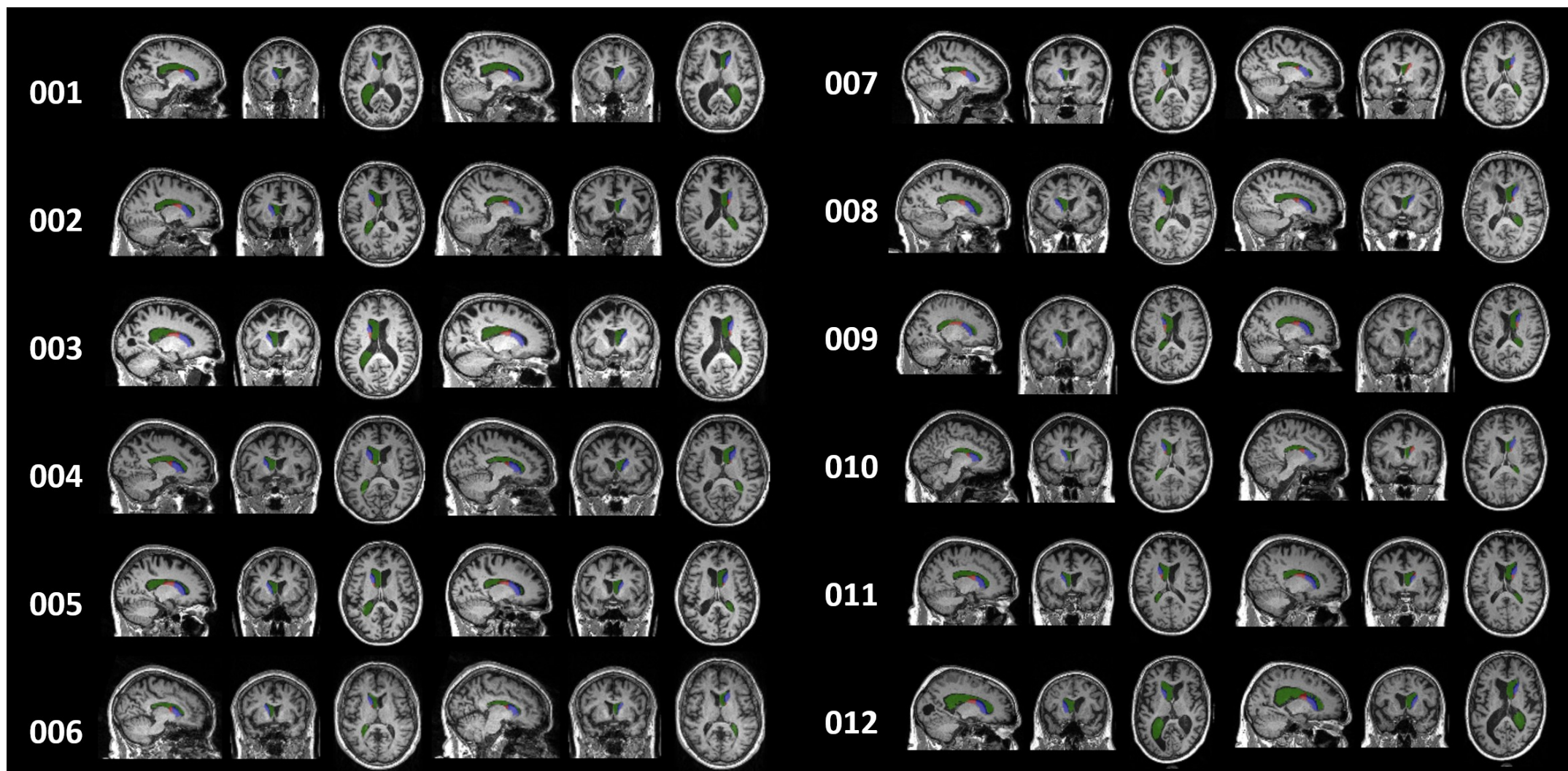


Figure S4.6: Figure showing good fits of caudate with CIC atlas overlaid on individual AD participant MRIs in native space for selected ROIs - pre-caudate (blue), post-caudate (red) and lateral ventricle (green).



Chapter 5

Imaging synaptic microstructure and synaptic loss *in vivo* in early Alzheimer's Disease

Abstract: Synaptic loss and neurite dystrophy are early events in Alzheimer's Disease (AD). To assess this *in vivo*, MRI neurite orientation dispersion and density imaging (NODDI) and diffusion tensor imaging (DTI) were used to image cortical microstructure in both sporadic, late onset, amyloid PET positive AD patients and healthy controls (total n = 28). I derived NODDI measures of grey matter extracellular free water (FISO), neurite density (NDI) and orientation dispersion (ODI), which provides an index of neurite branching and orientation, as well as more conventional DTI measures of fractional anisotropy (FA), mean/axial/radial diffusivity (MD, AD, RD, respectively). I also performed [¹¹C]UCB-J PET, which provides a specific measure of the density of pre-synaptic vesicular protein SV2A. Both sets of measures were compared to regional brain volumes. The AD patients showed expected relative decreases in regional brain volumes (range, -6 to -23%) and regional [¹¹C]UCB-J densities (range, -2 to -25%). Differences between AD and controls were greatest in the hippocampus. NODDI microstructural measures showed greater FISO (range, +26 to +44%) in AD, with little difference in NDI (range, -1 to +7%) and mild focal changes in ODI (range, -4 to +3%). Regionally greater FISO and lower [¹¹C]UCB-J binding were correlated across grey matter in patients (most strongly in the caudate, $r^2 = 0.37$, $p = 0.001$). FISO and DTI RD were

strongly positively associated, particularly in the hippocampus ($r^2 = 0.98$, $p < 7.4 \times 10^{-9}$). After 12-18 months I found a 5% increase in FISO in the temporal lobe, but little change across all ROIs in NDI and ODI. An exploratory analysis showed higher parietal lobe FISO was associated with lower language scores in people with AD. I interpreted the increased extracellular free water as a possible consequence of glial activation. The dynamic range of disease-associated differences and the feasibility of measuring FISO on commercially available imaging systems makes it a potential surrogate for pathology related to synapse loss that could be used to support early-stage evaluations of novel therapeutics for AD.

INTRODUCTION

Alzheimer's Disease (AD) is an ultimately fatal brain disease associated with progressive cognitive, social and functional decline. AD affects 50 million people worldwide. (2) The number of people affected will triple by 2050 without effective prevention strategies or treatments.(4)

AD begins with biochemical pathology, neuroinflammatory responses involving both microglial and astrocyte activation, and the deposition of amyloid- β ($A\beta$) (23,37,38) and tau aggregates.(159,160) Whilst substantial progress has been made in defining the protein pathology and the clinical phenotype, the pathogenesis, timing and distribution of early synaptic microstructural changes in humans are still poorly understood.

Most knowledge of synaptic pathology in AD has emerged from preclinical models or studies of *post mortem* human brain tissue. Clinical-pathological correlations suggest a strong relationship between loss of synaptic integrity and cognitive dysfunction (40,46). One model of AD pathogenesis proposes that amyloid oligomers induce maladaptive microglial synaptic pruning that leads to dendritic and axonal degeneration. (39,41,47) This loss of neuropil is believed to be a major contributor to the loss of brain volume as the disease progresses(31,32).

In vivo characterisation of early microstructural changes in AD complements neuropathological studies of *post mortem* human tissues and AD-related preclinical models. Informative and clinically practical measures of microstructural changes and synaptic loss promises earlier diagnosis and improved monitoring of disease progression and responses to new disease modifying therapies. (161)

Neurite Orientation Dispersion and Density Imaging (NODDI) is an advanced approach to *in vivo* diffusion-weighted MRI based on a multi-compartment diffusion model which allows a more precise characterisation of water diffusion than diffusion tensor imaging (DTI). (92) When applied to brain grey matter, NODDI provides measures related to the *extracellular* free water volume fraction (FISO), neurite density (NDI) (the *intracellular* volume fraction), and the local diffusion tensor orientation dispersion (ODI) (interpreted in this context as a measure of neurite branching). [¹¹C]UCB-J PET is a novel PET radioligand targeting the pre-synaptic vesicle glycoprotein 2A (SV2A) that allows the assessment of synaptic density *in vivo* in humans (28) (74) (75). Here I used

NODDI MRI and [^{11}C]UCB-J PET methods together to assess the microstructural pathology in AD and to explore its relationship to measures of clinical disease expression. I studied patients with sporadic, late onset AD and a comparable healthy control population.

RESULTS

Subject characteristics

28 participants contributed data to this study. 12 participants had a diagnosis of sporadic onset amnesic, amyloid-positive early-stage AD and 16 were healthy controls (Table 5.1).

Table 5.1

Cross-sectional demographic descriptive data and neuropsychological test results for AD patients and controls at baseline.

	AD (n=12)		CN (n=16)		p-value ¹
	Mean	SD	Mean	SD	
Age (Years)	75.50	7.82	65.88	9.82	0.008
MMSE	24.33	3.45	28.75	1.06	0.001
Immediate Memory	79.75	23.08	105.62	11.12	0.003
Visuospatial	101.83	17.54	98.56	9.61	0.568
Language	82.67	11.80	103.31	11.39	0.000
Attention	94.58	19.75	106.88	11.06	0.070
Delayed Memory	65.92	26.66	102.38	10.46	0.001
NART	36.67	8.63	35.94	10.85	0.845
¹ Unpaired two-tailed t-test assuming unequal variance					

One NODDI scan in each group could not be processed due to inability of the participant to complete the full scan, so 11 NODDI scans were available for assessment in the AD group and 15 for the controls. For DTI 11 scans were available in the AD group and 16 for controls. Both groups had similar premorbid intelligence as measured by the NART with equal distributions of males and females. The AD patients had a lower MMSE, immediate memory, language and

delayed memory test scores than the controls (Table 5.1). 8 participants with early AD were able to complete both baseline and 12-18 month follow-up SV2A PET scans and 10 patients were able to complete follow-up structural and NODDI MRI scans.

Brain volume and synaptic density are both reduced in early AD

All AD patient brain ROIs had lower volumes than those of the healthy controls. The greatest relative differences were in the hippocampus (mean -23%, median -18%, range -48% to -2%, $p < 0.001$) and temporal lobe grey matter (mean -16%, median -18%, range -33 to +5%, $p < 0.001$) (Figure 5.1, Supplementary Table S5.1).

[^{11}C]UCB-J DVR_{CS-1} was lower in brains of the AD patients than in controls (Figure 5.2). The greatest relative differences in SV2A density were found in the caudate (mean -25%, median -28%, range -75 to +16%, $p=0.007$), hippocampus (mean -24%, median -23%, range -52 to +6%, $p=0.001$) and thalamus (mean -19%, median -20%, range -44 to +21%, $p=0.012$) (Figure 5.2, Supplementary Table S5.1).

Figure 5.1

Cross-sectional percentage differences for individual AD patient (with group mean values and standard deviations) relative to the control mean for NDI, ODI, FISO, SV2A and normalised brain volume measures. Individual measures are provided as Supplementary Table S5.1

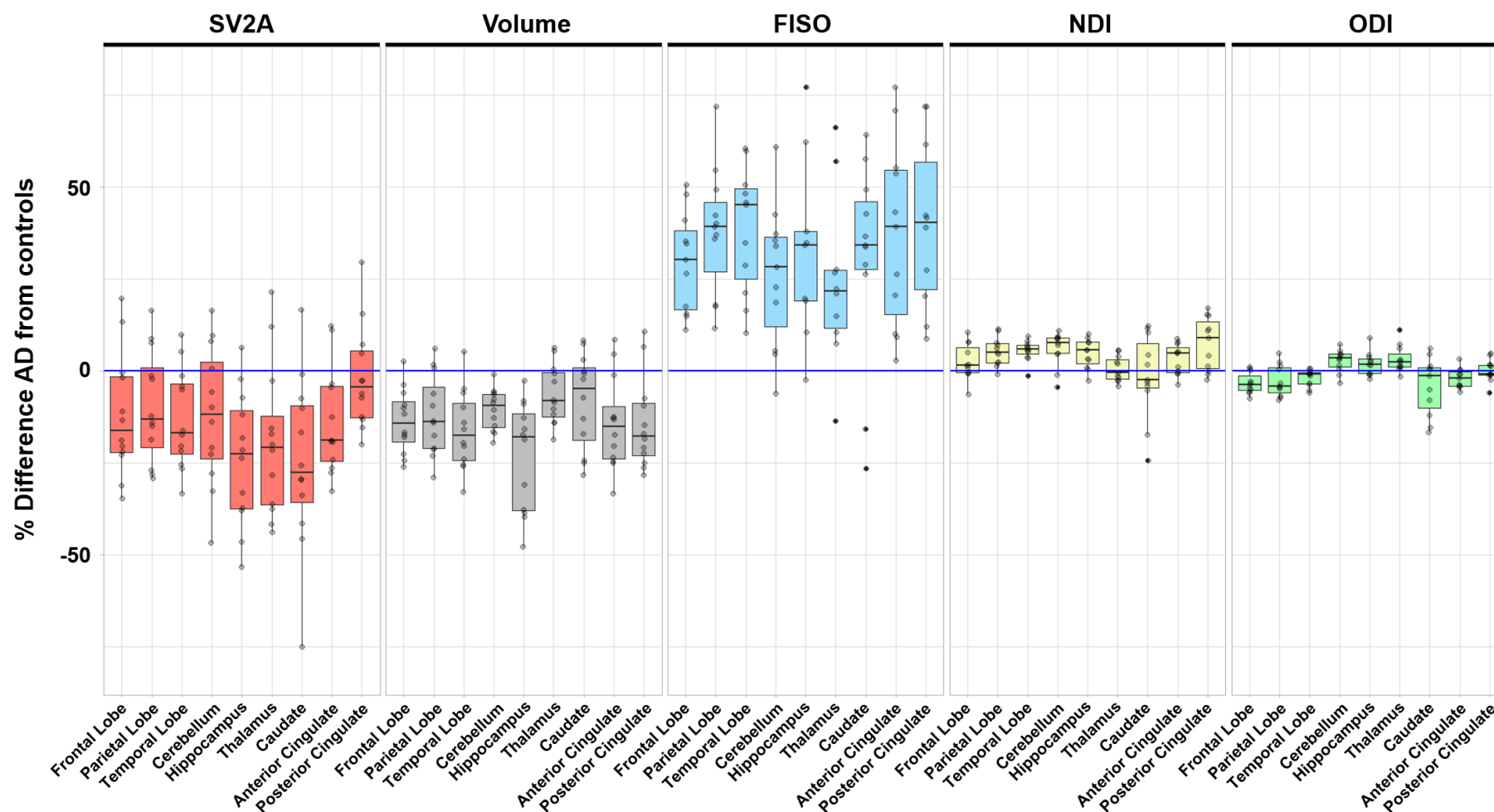
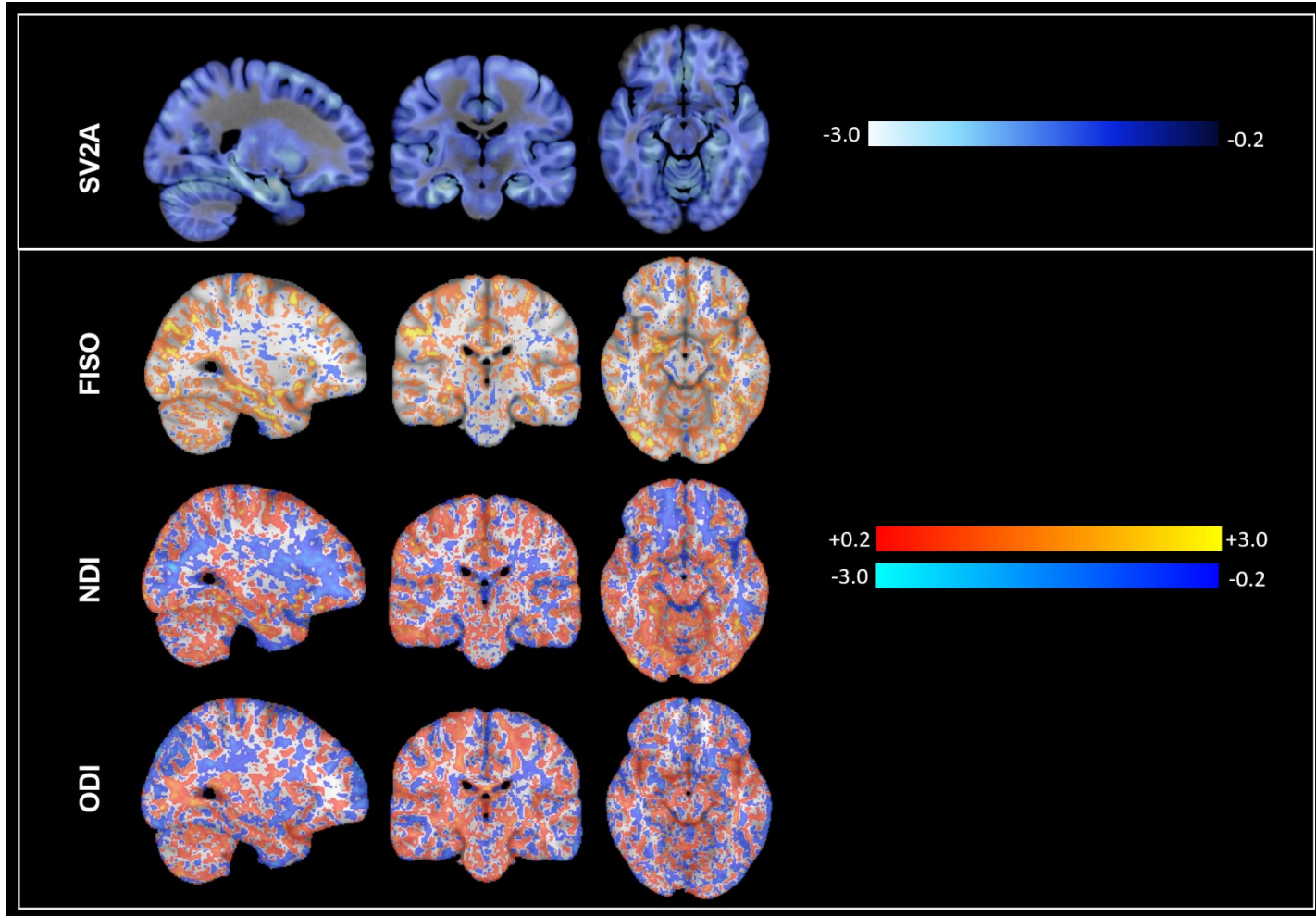


Figure 5.2

AD z score images on MNI152 for [^{11}C]UCB-J DVR_{CS-1} PET (SV2A) and NODDI images of free water (FISO) neurite density (NDI) and orientation dispersion (ODI) (top of image to bottom). Spatially normalised AD patient images acquired at the study baseline were contrasted to those from the control group. Thresholded voxel-wise z-scores ($((\text{AD patients mean} - \text{controls mean})/(\text{controls standard deviation}))$) are illustrated, using the group mean control image as a template. Z-score scales are shown to the right of the images.

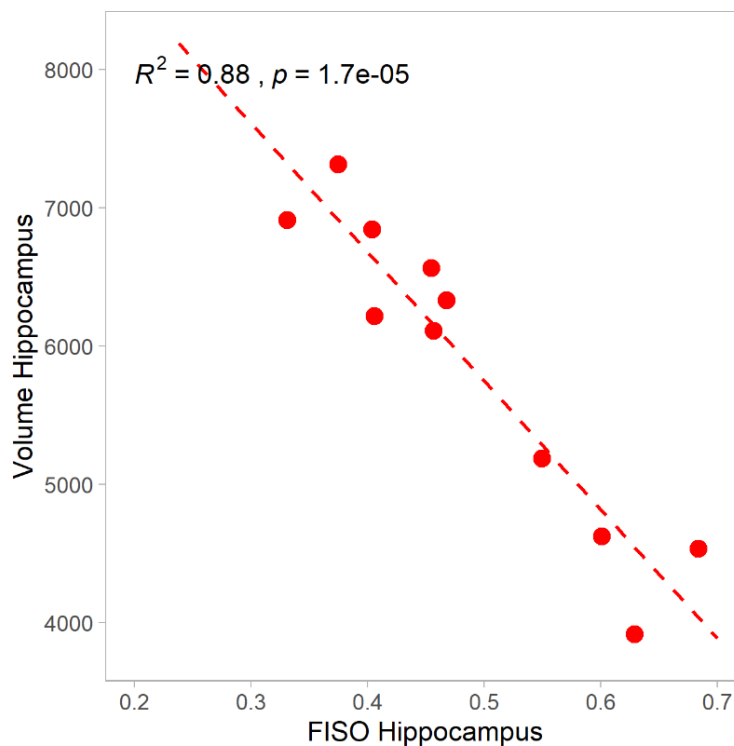


Cortical free water is increased in AD

NODDI FISO was higher in AD compared to the controls in all brain grey matter brain regions (range +26 to +44%) (Figure 5.1). The greatest relative differences in FISO were observed in the hippocampus (mean +44%, range -2 to 102%, $p = 0.001$) and posterior cingulate (mean +44%, range 9 – 91%, $p < 0.001$) (Figure 5.2, Supplementary Table S5.1). The strongest negative association between regional brain volume and FISO in the AD brains was found for the hippocampus ($r^2 = 0.88$, $p < 0.0001$) (Figure 5.3).

Figure 5.3

Highly significant negative associations between the normalised hippocampal volume and regional FISO in AD patients were found.



NDI values were also larger in the brains of the AD patients compared with the controls, but the relative differences were smaller than those observed with FISO (Figure 5.2 and Supplementary Table S5.1). The greatest increases in NDI with AD were in the parietal (mean +5%, range -1 to +12%, $p = 0.007$) and temporal (mean +5%, range -1 to +9%, $p < 0.001$) lobes, cerebellum (mean +6%, range -4 to +10%, $p = 0.002$), hippocampus (mean +5%, range -3 to +10%, $p = 0.045$) and posterior cingulate (mean +7%, range -2 to +17%, $p = 0.011$).

The ODI values were nominally lower in the AD patient brains compared to controls for some ROIs, but the magnitudes of the differences were small (Figure 5.2, Supplementary Table S5.1). The largest differences were found in the frontal (mean -3%, range -8 to +1%, $p = 0.007$) and medial temporal lobes (mean -2%, range -6 to 0%, $p = 0.038$).

I compared cross-sectional effect sizes between AD patients and controls for FISO, SV2A PET, and regional volumes using Hedges g (Table 5.2). The effect sizes (Hedge's g) were higher for FISO (range 0.82 - 2.30) than for the other NODDI and DTI measures in most ROIs and, notably, in the temporal lobe.

Table 5.2

Cross-sectional effect sizes using Hedges *g* for differences in SV2A PET, volume, NODDI FISO, NDI, ODI, and DTI FA, MD, L1 and RD measures for the AD patients compared to controls.

ROI	Effect sizes (Hedges <i>g</i>)								
	SV2A	Volume	FISO	NDI	ODI	FA	MD	L1	RD
Frontal Lobe	-0.80	-1.33	1.67	0.54	-1.19	0.43	1.50	1.58	1.43
Parietal Lobe	-0.69	-1.19	1.82	1.09	-0.65	0.20	1.69	1.69	1.69
Temporal Lobe	-1.12	-1.54	2.30	1.70	-0.89	-0.54	1.89	1.95	1.86
Cerebellum	-0.82	-1.60	1.35	1.44	0.74	-0.69	1.43	1.48	1.39
Hippocampus	-1.52	-1.97	1.66	0.75	0.55	-1.18	1.37	1.38	1.37
Thalamus	-1.07	-0.80	1.05	0.09	0.64	-1.00	0.77	0.74	0.78
Caudate	-1.17	-0.60	0.82	-0.13	-0.49	-1.12	0.69	0.61	0.73
Anterior Cingulate	-1.06	-1.16	1.54	0.66	-0.76	-0.22	1.32	1.36	1.29
Posterior Cingulate	-0.16	-1.16	1.66	1.12	0.02	-0.15	1.35	1.42	1.30

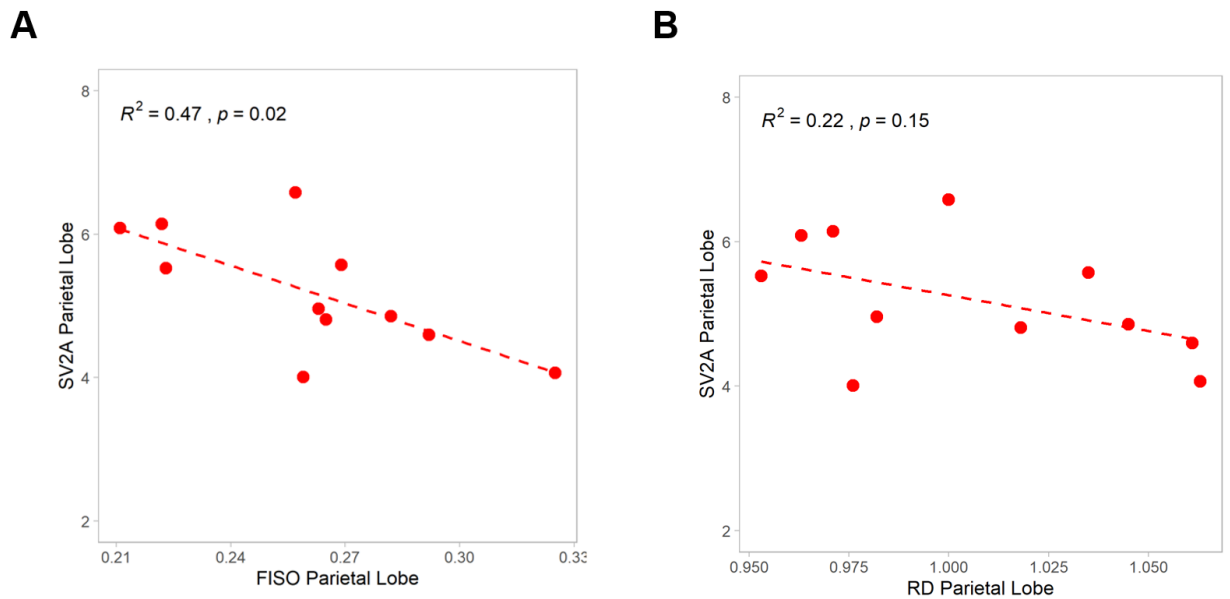
Lower SV2A synaptic density correlates strongly with increased grey matter extracellular free water

I tested for relationships between SV2A and NODDI measures (Figure 5.4A). I found negative correlations between SV2A and FISO in AD across multiple ROIs, particularly in the parietal lobe ($r^2 = 0.47$, $p < 0.02$), and for both AD patients and controls in the caudate ($r^2 = 0.37$, $p < 0.001$) and parietal lobe ($r^2 = 0.21$, $p = 0.018$).

Weaker correlations for AD and controls were found between SV2A and NDI, but these were present only for a subset of the grey matter ROIs, such as the frontal ($r^2 = 0.17$, $p < 0.041$) and parietal ($r^2 = 0.33$, $p < 0.0028$) lobes (but not, e.g., in the hippocampus, $r^2 = 0.022$, $p = 0.47$). Similarly, SV2A and ODI were weakly correlated in only some ROIs (e.g., the parietal lobe, $r^2 = 0.15$, $p < 0.049$ and caudate, $r^2 = 0.2$, $p < 0.025$).

Figure 5.4

A negative association between SV2A and FISO (A) and a trend to a negative association for SV2A and RD (B) in the parietal lobe in AD were found.



Higher DTI RD correlates strongly with NODDI FISO in brain grey matter

DTI RD was globally significantly higher in AD patient brain grey matter than in that of the controls (range +6 to 25%). The greatest relative differences were in the hippocampus (mean +25%, range -3 to 70%, $p = 0.007$), cerebellum (mean +11%, range -0.6 to 24%, $p = 0.001$), parietal (mean +44%, range -2 to 102%, $p = 0.001$) and temporal lobes (mean +9%, range -1 to 16%, $p < 0.001$). DTI MD and L1 results showed similar relative differences (see Supplementary Figure S5.1 and Supplementary Table S5.2 for the full DTI results). I found strong positive associations between regional NODDI FISO and DTI RD (Supplementary Figure 5.2) across all ROIs in the AD patients, particularly for the parietal lobe ($r^2 = 0.81$, $p < 0.001$) and hippocampus ($r^2 = 0.98$, $p < 7.4 \times 10^{-9}$). The effect sizes for DTI RD were lower than for NODDI FISO across all ROIs.

DTI FA was lower in grey matter of brains of AD patients compared to controls only in some ROIs (range -12 to +2%). The greatest relative differences were observed in the hippocampus (mean -12%, range -30 to 5%, $p = 0.006$) and thalamus (mean -6%, range -19 to +2%, $p < 0.023$) and caudate (mean -9%, range -21 to +2%, $p = 0.006$) (Supplementary Figure S5.1, Supplementary Table S5.2). DTI RD showed a nominal trend towards negative correlations with SV2A, particularly in the parietal lobe in AD patients ($r^2 = 0.22$, $p = 0.15$) (Figure 5.4B).

Longitudinal changes in AD brains

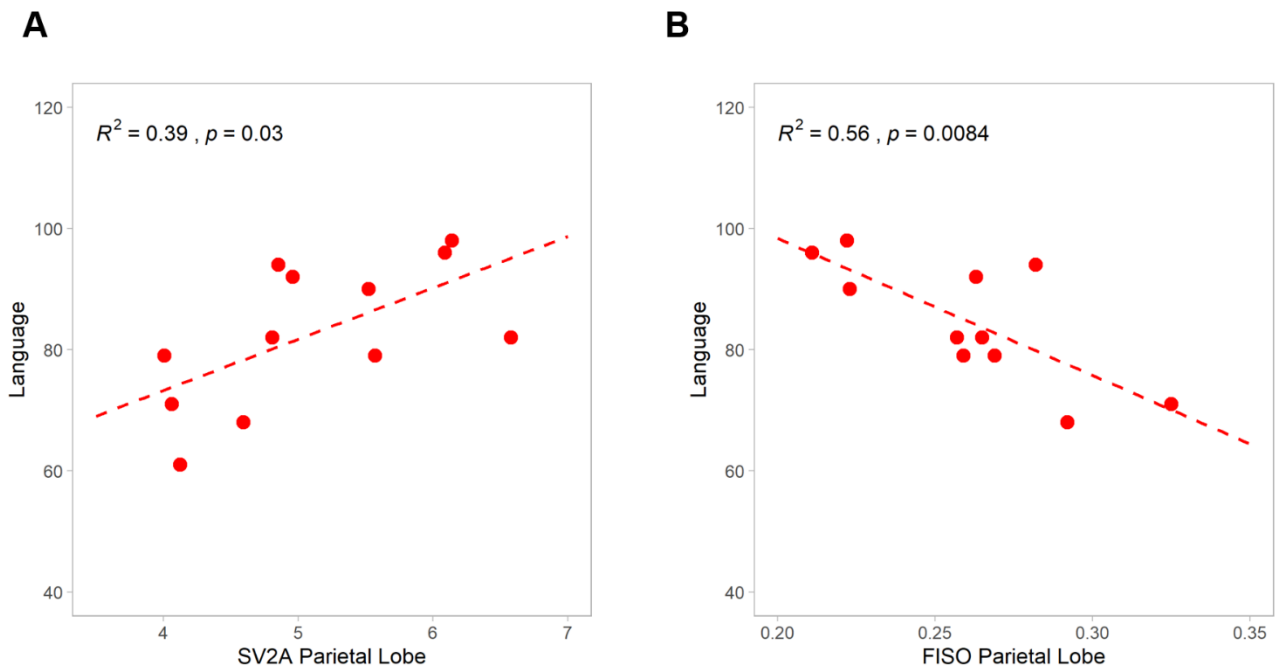
I re-scanned the AD group after 12-18 months (see Supplementary Table 3). I found small global decreases in brain volume at follow up, with greater relative volume changes in the temporal lobe (-4%, $p=0.009$), hippocampus (-5%, $p=0.01$), parahippocampal gyrus (-3%, $p=0.027$), cingulate cortex (-3%, $p=0.023$) and cerebellum (-2%, $p=0.020$). These were accompanied by small increases in FISO, significant in the medial temporal lobe (+5%, $p = 0.046$). I did not find any change in NDI and, although there were small reductions in mean ODI in the frontal lobe (-1%, $p = 0.029$) and in other ROIs, they were not statistically significant. I also found small and non-significant reductions in [^{11}C]UCB-J $\text{DVR}_{\text{CS-1}}$ after 12-18 months.

Imaging associations with cognition

While my study was not well powered to test for relationships between the imaging and cognitive performance measures, I explored relationships amongst PET SV2A density and MRI microstructural imaging measures in the AD patients and controls. Lower regional SV2A was associated with impaired language (picture naming and semantic fluency) in the AD patients (hippocampus, $r^2 = 0.68$, $p=0.0001$, parietal lobe, $r^2 = 0.39$, $p=0.03$), but not in controls. Higher FISO in the parietal lobe was associated with impaired language in AD patients ($r^2 = 0.56$, $p=0.0084$), but not in controls ($r^2 = 0.081$, $p=0.3$) (Figure 5.5).

Figure 5.5

Loss of SV2A (A) and higher FISO (B) both were associated with impaired language (picture naming and semantic fluency) in the parietal lobe of AD patients



DISCUSSION

I combined diffusion MRI and synaptic PET imaging to map changes in grey matter microstructure to characterise relationships between diffusion MRI measures and synaptic loss in early AD. Consistent with other recent reports, my study showed large decreases in SV2A density as well as brain atrophy in people with early AD. I also discovered striking increases in extracellular free water (FISO) in grey matter and consistent negative correlations between grey matter FISO and co-localised measures of both SV2A density and regional brain volume. FISO had a greater

effect size for detecting differences between AD and controls in most ROIs than did the PET SV2A measures, regional volumes or the other NODDI and DTI indices; NDI and ODI showed only small relative changes in the AD brains relative to the healthy controls. I also found strong associations of FISO with DTI RD in the grey matter. Over 12-18 months of follow up of the AD patients, I found significant atrophy and FISO increases in the temporal lobe, although little change was measured in NDI, ODI or SV2A density. Exploration of relationships of the MRI and PET SV2A measures with cognitive performance suggested associations of higher parietal lobe FISO with lower language performance and greater SV2A with higher language performance.

SV2A is abundant in the brain, where it plays a role in presynaptic neurotransmitter release through Ca^{2+} -dependent exocytosis (76) (77). [^{11}C]UCB-J was developed as a PET tracer to image the distribution and abundance of SV2A as an index of synaptic density (74,117,118). Previous studies have shown substantial reductions of SV2A binding in medial temporal and neocortical brain regions in early AD (119,120,162). I observed differences of similar sizes and distributions, even after correction of the [^{11}C]UCB-J DVR for relative local tissue atrophy. (119,162)

My most novel finding was of large increases in regional cortical FISO with AD that were inversely correlated with SV2A density. I interpret the increased FISO as an index of the glial activation(163,164); glial water and the extracellular matrix changes together can modulate water diffusivity in the extracellular space. (165) Microglial and astrocyte activation are prominent in early AD. (160,166,167)

Phagocytic microglial activation and pro-inflammatory astrocyte activation may be responsible for the pathological synaptic loss in AD. (168,169) An additional contribution may arise from changes in cortical mini-column organisation, particularly in later stages of AD. (170)

While there also may be a contribution from pathological loss of neuronal organisation, I believe that the small increases in neurite density and relative reductions in ODI, together with large FISO increases, are consistent with a model in which early AD neuropathology is dominated by synaptic loss, associated glial activation and a consequent reduction in the complexity of neuritic arborisation. While I observed small relative increases in neurite density (NDI), the latter could be a consequence of changes in neuronal organisation with distal neurite atrophy and relatively preserved proximal segments (171,172) or an adaptive increase in dendritic growth. (173)

The vast majority of diffusion imaging studies in AD have focused on white matter, (174,175) although changes in grey matter mean diffusivity have recently begun to be explored (163) Contributions of an explicitly-modelled free water compartment in AD have begun to be elucidated in the white matter, but the role of free water, and more sophisticated diffusion models more generally, have not previously been developed explicitly for grey matter. To date, NODDI has been applied in only a small number of human studies along the AD continuum, e.g., with healthy aging in the UK Biobank, (176) in mild cognitive impairment, (177,178) young onset AD (174,179,180), and sporadic late-onset AD. (175,181) Of these, only two have assessed grey matter changes in AD using NODDI and neither of these reported

assessments of free water changes. One of these reports included data from people with early-onset, clinically defined AD that showed neurite density was lower in all ROIs compared to a healthy control group. Decreased ODI was found in inferior and middle temporal gyri, fusiform gyrus and precuneus, and cortical NDI was associated with cognitive impairment. (180) Another study explored correlations between grey matter changes in neurite density and tau PET in sporadic AD. (181) Decreased neurite density was associated regionally with tau deposition which is itself associated with neuroinflammation. Another, more recent study suggested FISO could provide an surrogate measure of tau deposition in AD. (182) Increased grey matter FISO was reported with a mouse model of tauopathy and in a recent human study of tau aggregate expression *in vivo*. (183) Although I found that the conventional DTI measures, which do not incorporate a microstructure compartmental model, also are sensitive to differences with disease (as reported by (170)), they have smaller effect sizes in the temporal lobe and hippocampus particularly, and have less specific pathological interpretations.

Despite limitations in size and scope, my study provides a foundation for characterising grey matter microstructure associated with synaptic loss and is the first to my knowledge to attempt this directly *in vivo*. While my study population was small, the combination of phenotyping depth with multi-modal imaging measures related to synaptic microstructure make it unique to my knowledge. The NODDI compartmental modelling appears informative but is interpreted using a simple model of grey matter structure and organisation that has not been validated quantitatively. This will demand new work well out of the scope of my

investigations. My study was not powered to test cognitive endpoints, so my tests for the clinical meaningfulness of the findings must be considered only as exploratory. Nonetheless, their general consistency with prior studies increases confidence in their potential generalisability. (119,163,182) Finally, although my AD patient and control groups were matched for sex, there was a 10 year mean age difference and both synaptic density and total GM volume decrease with aging. (184,185) However, age-related changes alone are relatively small (186). In a larger cohort of 80 cognitively normal subjects (21-83 years old), the total brain GM volume was 2.9% lower per decade. Greatest age-related differences in GM SV2A density were reported in the caudate (6.4%/decade) (186). Similar relative regional differences in GM SV2A density were found in the full MINDMAPS cohort of healthy controls (20-80 years old, n=24, unpublished), although age-related changes in SV2A binding measured were even lower (e.g., 3.5%/decade in the caudate). Group differences in SV2A binding and volume between AD and healthy controls that I observed for the current study were 3 to 7-fold greater than these values, so I do not believe that the age difference between my control and AD groups has a substantial impact on interpretation of my results.

I have assessed the relationship between cortical microstructure and synapse loss *in vivo* in early AD for the first time. My results suggest that NODDI MRI, and changes in free water in particular, could be more sensitive to differences in cortical pathology related to synaptic loss in early AD than the more molecularly-specific SV2A PET. Future work should both further test the cross-sectional associations I have reported and the relative sensitivity of these measures to longitudinal disease

progression. These imaging methods and their extension as longitudinal measures have the potential to contribute to the better understanding of early synaptic vulnerability and homeostasis. My observations suggest that NODDI MRI could be a useful adjunct to MRI measures of regional cortical volume change for stratification and monitoring of disease progression in people with AD.

SUPPLEMENTARY MATERIAL

Supplementary Table 1

Baseline neurite density (NDI), orientation dispersion (ODI), free water (FISO), [^{11}C]UCB-J DVR_{CS-1} (synaptic density) with partial volume correction, and brain volume measures in AD patients and controls.

Cross-sectional results of NODDI (NDI,ODI & FISO), SV2A & volume in AD and controls																																			
ROI	NODDI NDI							NODDI ODI							NODDI FISO							[11C]UCB-J DVRCS – with PVC							Volume						
	AD mean	sd	CN mean	sd	% AD from CN	p-value ¹	adjusted p-value ²	AD mean	sd	CN mean	sd	% AD from CN	p-value ¹	adjusted p-value ²	AD mean	sd	CN mean	sd	% AD from CN	p-value ¹	adjusted p-value ²	AD mean	sd	CN mean	sd	% AD from CN	p-value ¹	adjusted p-value ²	AD mean	sd	CN mean	sd	% AD from CN	p-value ¹	adjusted p-value ²
Frontal Lobe	0.464	0.022	0.452	0.019	2	0.188	0.241	0.455	0.014	0.470	0.012	−3	0.007	0.061	0.204	0.022	0.157	0.030	30	0.000	0.000	5.13	0.98	5.82	0.73	−12	0.052	0.079	699	71	809	86	−14	0.001	0.002
Parietal Lobe	0.472	0.018	0.448	0.023	5	0.007	0.021	0.457	0.020	0.470	0.017	−3	0.113	0.169	0.261	0.033	0.189	0.041	38	0.000	0.000	5.11	0.87	5.65	0.67	−10	0.089	0.100	810	102	923	84	−12	0.005	0.007
Temporal Lobe	0.545	0.015	0.517	0.017	5	0.000	0.001	0.480	0.011	0.490	0.009	−2	0.038	0.153	0.226	0.028	0.163	0.025	38	0.000	0.000	4.65	0.73	5.34	0.49	−13	0.010	0.024	991	128	1181	113	−16	0.000	0.001
Cerebellum	0.688	0.031	0.649	0.023	6	0.002	0.010	0.472	0.014	0.459	0.018	3	0.059	0.153	0.261	0.040	0.208	0.037	26	0.002	0.003	2.81	0.61	3.19	0.31	−12	0.061	0.079	860	54	961	66	−11	0.000	0.001
Hippocampus	0.621	0.024	0.593	0.042	5	0.045	0.081	0.459	0.014	0.450	0.016	2	0.156	0.200	0.487	0.113	0.339	0.061	44	0.001	0.002	2.15	0.52	2.82	0.36	−24	0.001	0.009	58	11	75	6	−23	0.000	0.001
Thalamus	0.684	0.024	0.680	0.052	1	0.790	0.790	0.346	0.012	0.335	0.019	3	0.087	0.156	0.340	0.077	0.262	0.069	30	0.015	0.016	2.96	0.76	3.66	0.53	−19	0.013	0.024	193	17	206	15	−6	0.043	0.049
Caudate	0.567	0.067	0.575	0.056	−1	0.739	0.790	0.408	0.034	0.425	0.035	−4	0.210	0.237	0.377	0.081	0.290	0.116	30	0.033	0.033	2.76	0.88	3.68	0.66	−25	0.007	0.024	43	6	47	6	−8	0.114	0.114
Anterior Cingulate	0.507	0.021	0.492	0.025	3	0.090	0.135	0.460	0.013	0.470	0.011	−2	0.068	0.153	0.169	0.031	0.124	0.027	37	0.001	0.002	5.23	0.90	6.06	0.65	−14	0.013	0.024	240	36	280	32	−14	0.006	0.007
Posterior Cingulate	0.525	0.035	0.489	0.028	7	0.011	0.025	0.459	0.014	0.458	0.014	0	0.957	0.957	0.224	0.042	0.155	0.039	44	0.000	0.001	4.97	0.72	5.07	0.40	−2	0.687	0.687	90	13	106	12	−14	0.005	0.007
¹ Unpaired two-tailed t-test assuming unequal variance																																			
² False Discovery Rate																																			

Supplementary Table 2

Baseline DTI measures for FA, MD, L1, RD in AD patients and controls.

Cross-sectional results of DTI (FA, MD, L1, RD) in AD and controls																												
ROI	FA							MD							L1							RD						
	AD mean	sd	CN mean	sd	% AD from CN	p-value ¹	adjusted p-value ²	AD mean	sd	CN mean	sd	% AD from CN	p-value ¹	adjusted p-value ²	AD mean	sd	CN mean	sd	% AD from CN	p-value ¹	adjusted p-value ²	AD mean	sd	CN mean	sd	% AD from CN	p-value ¹	adjusted p-value ²
Frontal Lobe	0.16	0.01	0.15	0.01	2	0.303	0.454	1.011	0.027	0.953	0.043	6	0.000	0.001	1.149	0.029	1.086	0.044	6	0.000	0.000	0.941	0.027	0.887	0.042	6	0.000	0.001
Parietal Lobe	0.15	0.01	0.15	0.01	1	0.632	0.703	1.076	0.043	0.992	0.051	8	0.000	0.001	1.215	0.049	1.123	0.055	8	0.000	0.000	1.006	0.040	0.927	0.049	9	0.000	0.001
Temporal Lobe	0.15	0.01	0.16	0.01	-2	0.173	0.311	1.016	0.048	0.935	0.036	9	0.000	0.001	1.155	0.050	1.070	0.036	8	0.000	0.000	0.947	0.048	0.867	0.037	9	0.000	0.001
Cerebellum	0.18	0.01	0.19	0.02	-6	0.067	0.151	0.931	0.057	0.849	0.055	10	0.001	0.003	1.092	0.058	1.009	0.052	8	0.001	0.002	0.851	0.058	0.768	0.057	11	0.001	0.003
Hippocampus	0.15	0.02	0.17	0.02	-12	0.006	0.027	1.520	0.278	1.235	0.126	23	0.007	0.009	1.725	0.284	1.435	0.124	20	0.007	0.010	1.418	0.276	1.135	0.128	25	0.007	0.009
Thalamus	0.32	0.02	0.34	0.02	-6	0.023	0.068	1.177	0.190	1.037	0.167	14	0.062	0.066	1.496	0.187	1.361	0.170	10	0.070	0.079	1.017	0.191	0.874	0.166	16	0.059	0.059
Caudate	0.21	0.02	0.23	0.02	-9	0.006	0.027	1.327	0.185	1.165	0.254	14	0.066	0.066	1.549	0.203	1.387	0.290	12	0.100	0.100	1.216	0.177	1.054	0.236	15	0.052	0.059
Anterior Cingulate	0.17	0.01	0.17	0.01	-1	0.548	0.703	0.964	0.035	0.916	0.036	5	0.002	0.003	1.110	0.037	1.061	0.034	5	0.002	0.003	0.891	0.035	0.843	0.037	6	0.002	0.003
Posterior Cingulate	0.16	0.01	0.16	0.01	-1	0.703	0.703	1.014	0.051	0.940	0.054	8	0.002	0.003	1.165	0.054	1.087	0.052	7	0.001	0.002	0.939	0.050	0.867	0.055	8	0.002	0.003
¹ Unpaired two-tailed t-test assuming unequal variance																												
² False Discovery Rate																												

Supplementary Table 3

Neurite density (NDI), orientation dispersion (ODI), free water (FISO), [^{11}C]UCB-J DVR_{CS-1} (synaptic density) with partial volume correction, and brain volume measures in AD patients after 12-18 month follow-up.

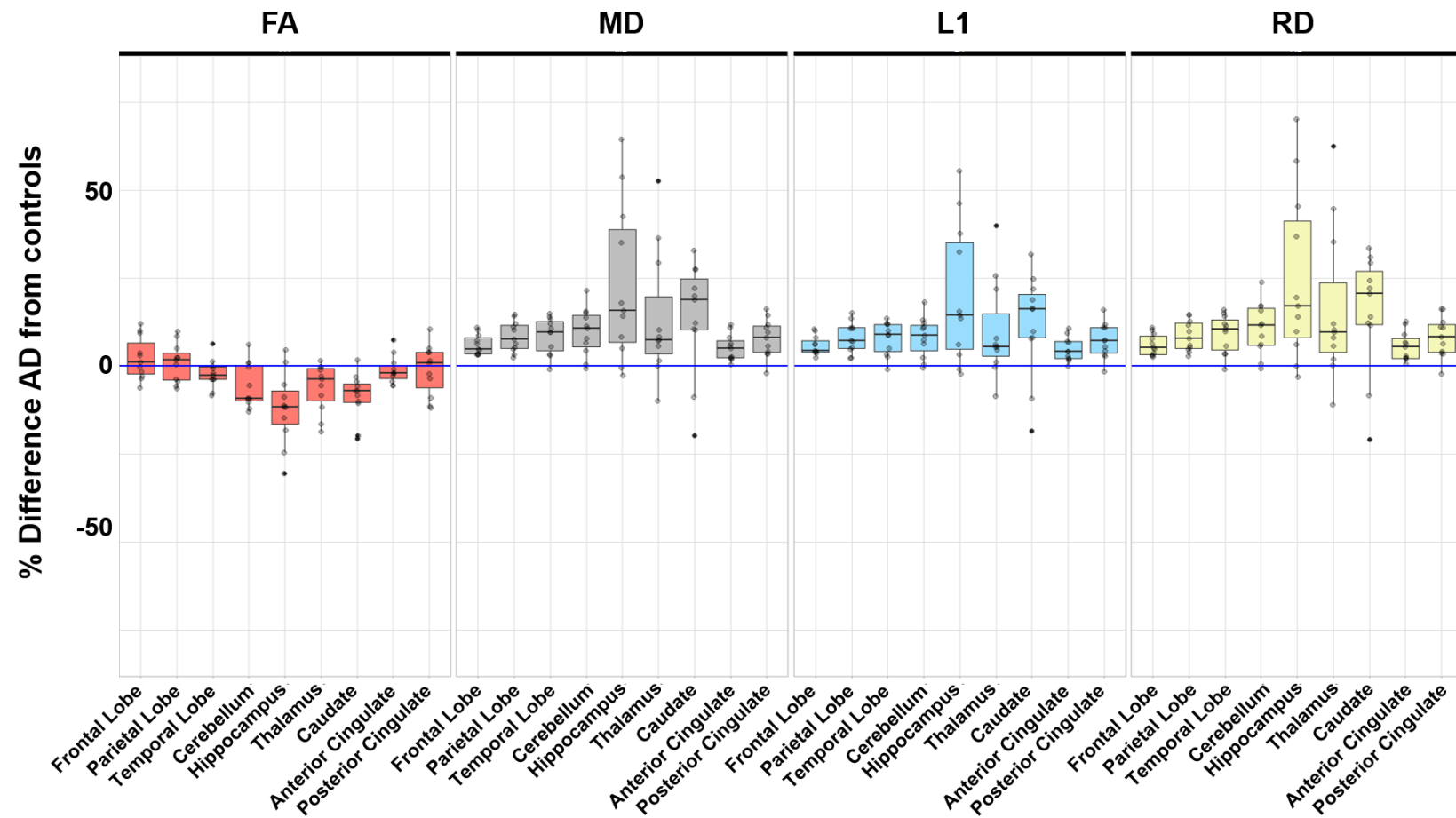
Longitudinal results of NODDI (NDI,ODI & FISO), SV2A & volume in AD and controls																																			
ROI	NODDI NDI						NODDI ODI						NODDI FISO						¹¹ C]UCB-J DVR _{CS} – with PVC						Volume										
	Baseline mean	sd	Longitudinal mean	sd	% from baseline	p-value ¹	adjusted p-value ²	Baseline mean	sd	Longitudinal mean	sd	% from baseline	p-value ¹	adjusted p-value ²	Baseline mean	sd	Longitudinal mean	sd	% from baseline	p-value ¹	adjusted p-value ²	Baseline mean	sd	Longitudinal mean	sd	% from baseline	p-value ¹	adjusted p-value ²	Baseline mean	sd	Longitudinal mean	sd	% from baseline	p-value ¹	adjusted p-value ²
Frontal Lobe	0.467	0.024	0.462	0.029	−1	0.257	0.967	0.454	0.013	0.447	0.017	−1	0.029	0.247	0.20	0.02	0.20	0.03	−0	0.876	0.876	5.00	1.00	4.93	1.25	−0	0.768	0.996	709	74	688	65	−3	0.208	0.262
Parietal Lobe	0.476	0.018	0.476	0.027	0	0.967	0.967	0.454	0.018	0.446	0.021	−2	0.098	0.293	0.27	0.03	0.27	0.04	3	0.268	0.402	5.13	0.89	5.02	1.07	−2	0.587	0.996	816	108	789	82	−3	0.224	0.262
Temporal Lobe	0.549	0.010	0.550	0.012	0	0.769	0.967	0.478	0.011	0.479	0.007	0	0.722	0.912	0.23	0.03	0.24	0.03	5	0.046	0.291	4.54	0.74	4.60	0.84	−3	0.855	0.996	1003	122	964	127	−4	0.009	0.045
Cerebellum	0.687	0.034	0.688	0.042	0	0.910	0.967	0.473	0.016	0.475	0.011	0	0.519	0.856	0.26	0.04	0.27	0.04	4	0.213	0.402	2.79	0.63	2.84	0.46	−1	0.770	0.996	875	46	857	49	−2	0.020	0.045
Hippocampus	0.620	0.024	0.617	0.027	−0	0.811	0.967	0.459	0.015	0.459	0.016	0	0.971	0.971	0.51	0.11	0.54	0.12	5	0.066	0.291	2.13	0.43	2.13	0.51	−2	0.996	0.996	58	11	55	11	−5	0.010	0.045
Thalamus	0.689	0.023	0.694	0.034	1	0.665	0.967	0.347	0.013	0.344	0.011	−1	0.570	0.856	0.35	0.08	0.35	0.09	2	0.458	0.589	2.99	0.81	2.95	1.02	−1	0.841	0.996	193	14	193	14	−0	0.623	0.623
Caudate	0.592	0.041	0.596	0.033	1	0.579	0.967	0.417	0.030	0.411	0.027	−2	0.055	0.247	0.37	0.09	0.39	0.09	5	0.097	0.291	2.67	1.00	2.83	1.06	1	0.652	0.996	43	5	42	5	−3	0.233	0.262
Anterior Cingulate	0.510	0.022	0.511	0.021	0	0.800	0.967	0.459	0.014	0.458	0.012	−0	0.811	0.912	0.17	0.03	0.17	0.03	2	0.707	0.795	5.09	0.88	5.10	0.98	−1	0.954	0.996	245	37	238	39	−3	0.018	0.045
Posterior Cingulate	0.531	0.036	0.533	0.047	0	0.763	0.967	0.457	0.013	0.451	0.018	−1	0.223	0.501	0.23	0.04	0.24	0.05	3	0.226	0.402	4.90	0.78	4.86	0.88	−1	0.839	0.996	92	14	90	15	−3	0.051	0.092

¹Paired two-tailed t-test assuming equal variance

²False Discovery Rate

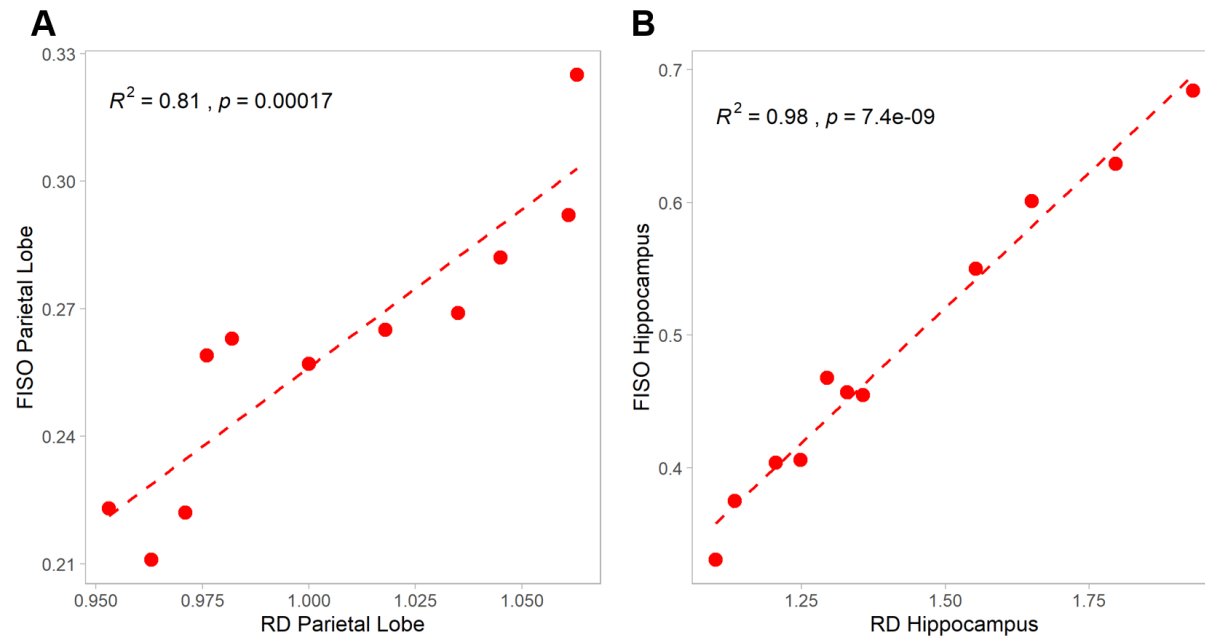
Supplementary Figure 1

DTI cross-sectional results for FA, MD, L1, RD with box and whisker plots of individual AD patient percentage differences from the mean controls corresponding with values in Supplementary Table 2.



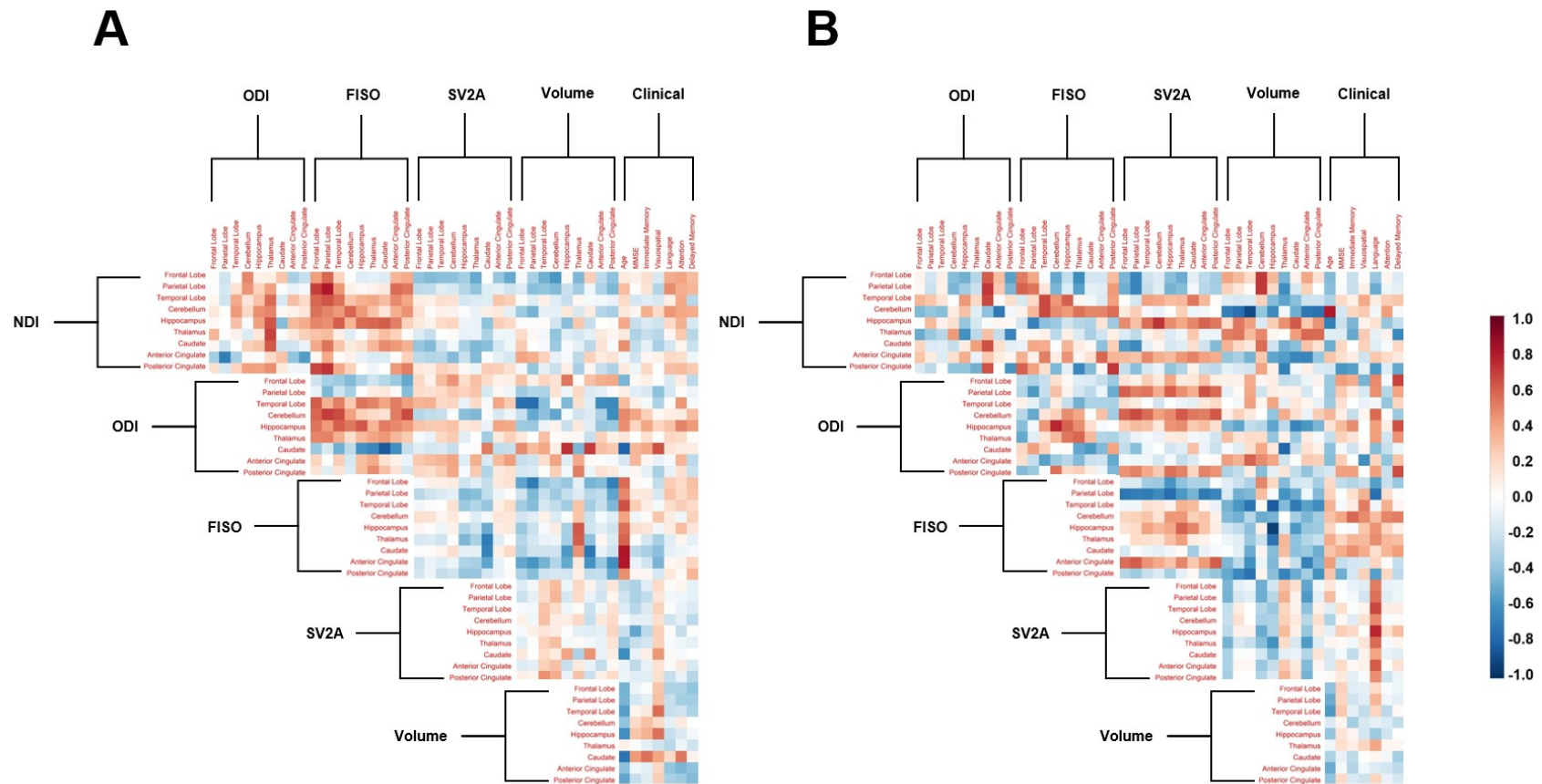
Supplementary Figure 2

Significant positive associations between regional FISO and RD in AD patients for the parietal lobe (A) and hippocampus (B) were found.



Supplementary Figure 3

Pearson's r correlation matrices describe correlations between regional mean neurite density (NDI), orientation dispersion (ODI), free water (FISO), SV2A ($[^{11}\text{C}]\text{UCB-J DVR}_{\text{CS-1}}$) with partial volume correction, normalised brain volume and clinical variables for controls (n=16) (A) and AD (n=12) patients (B). The scale for the Pearson's r correlation values is shown on the right.



Chapter 6

Summary and Future Directions

The cellular phase of AD ranges from brain protein deposition to cellular homeostatic collapse and multi-system failure over a long prodromal period. Together these culminate to form a devastating impact on the individual suffering from the disease, alongside their family and friends. Advances in brain imaging provide a unique way of assessing early pathology and longitudinal change in patients *in vivo*. This thesis has shown how imaging using PET accompanied by MR can lead to better diagnostic classification, and a better understanding of the neurobiology in order to prioritise targets for earliest interventions to delay or reverse disease.

The complex and multifactorial nature of AD presents significant challenges for precision medicine, both for diagnostic accuracy, patient monitoring and clinically meaningful disease modification. It is emerging that AD pathophysiology progression is non-linear and dynamic with multiple feedback loops (21,50) that occur beyond the more traditional linear cascades arising from protein deposition. An equilibrium exists, that in disease is slowly disrupted when various thresholds of failure of brain reserve (82) are surpassed. We lack critical aspects of knowledge

of the underlying disease process, specifically in how cell stress causes decompensation of cellular mechanisms that promote survival.

There is also an unmet need for more robust diagnostic methods that allow better stratification of patients in order to direct them to appropriate management, and provide prognostic information. Additionally for clinical trials for AD, there is a specific need to trial potential medications for disease modification and symptomatic treatments in the right patient population. It is increasingly recognised that this population is likely to be those early in their disease. (187) Given the variety of neuropsychiatric symptoms in AD, the varying trajectories, and sometimes daily fluctuations in symptom intensity and frequency, both doctors and patients would benefit from additional diagnostic certainty.

In AD therapeutics, there is a need for the discovery of both new targets for intervention that are clinically meaningful, and new biomarkers of disease that are sensitive to those clinical effects. At present of the 126 agents in clinical trials (188) for AD there are a variety of classes from potential disease modification, management of neuropsychiatric symptoms, and symptomatic cognitive enhancers. Despite this number, and that the number of potential agents are increasing, the problem of the heterogeneity of the AD pathological process at various time points in the course of the disease (if it is indeed one disease) are increasingly recognised.

Utilising spatial information of the behaviour of amyloid- β , in chapter 3, I was able to create an algorithm for automated decision support that boosted the diagnostic specificity using information from A β PET alone across 758 volunteers across the AD continuum. I was able to spatially constrain key areas of A β deposition using k-means clustering and test for their associations with cognition and diagnostic category. I was able to optimise a support vector machine to test a classifier that outperformed existing spatial methods and also explore disease staging by mapping the trajectory of disease. This approach has various strengths, including a large sample size across the AD continuum, an explainable machine learning model that prevented overfitting of the data, and captured variances across multiple sites. In addition a data driven approach was not limited by the reduced degree of precision of atlas based approaches or visual reads. By exploring clinical associations, I was further able to support a level of diagnostic classification not limited by visual reads using an automated data driven spatially informed classifier.

There were limitations of this approach to diagnose, as discussed in chapter 3. Taking these further amidst the broader field, one of the main premises of this study is that amyloid- β is associated with cognition, and in the wider literature deposition is seen to be necessary but not sufficient to cause disease. (50). In order to assess the generalisability of these findings they would need testing and replication in other specific populations of different ethnic and national groups. Since this study was conducted, approaches to classification have progressed beyond PET and amyloid- β . For example there are clear correlates of disease with tau PET (51,136)

that have emerged since this study but are not yet widely available. In addition, moving forward with diagnostic classifications blood based biomarkers (validated by either CSF or PET) have recently made breakthroughs and are acceptable and scalable for stratifying of disease. However, they have not yet been validated and approved clinically, and are unlikely to replace the certainties associated with imaging.

In chapter 4, I showed new evidence for widespread cell stress and bioenergetic mitochondrial dysfunction in early AD, with exploratory associations showing that they these may indeed be clinically meaningful. This was the most novel finding in my thesis as unlike previously described AD hypotheses my results are consistent with a disease model in which generalised cell stress leads to regionally variably expressed pathologies of mitochondrial dysfunction, synaptic loss and clinical expression of AD. I was able to quantify density of the ER stress marker, the sigma 1 receptor, and showed widespread increases in AD compared to controls. I showed widespread decreases in regional mitochondrial complex I, and synaptic density, alongside reduced cerebral blood flow, and brain volume. In following the AD patients after 12-18 months I showed larger decreases in MC1 density. I interpret this data as suggesting a loss of functional metabolic reserve with AD, with specific clinical associations with MC1, SV2A and S1R density and cognition. This study was unique for its *in vivo* and longitudinal characterisation of the molecular markers relating to cell stress, mitochondrial and synaptic dysfunction in AD. I found a generalised cell stress response with focal decreases highlighting regional

vulnerability to pathology particularly in the hippocampus but also the thalamus and caudate. I also highlighted the utility of MC1 PET for assessing longitudinal change in this group of patients.

One of the limitations of this approach other than described in Chapter 4 is the degree of validation of the relationship of the sigma 1 receptor density as a marker of cell stress in this specific disease context. However currently *in vivo* there are no other ways to visualise this *in vivo* other than using this PET tracer. An obvious question that arises is whether, if the sigma 1 receptor was modified, this would change disease course? Assuming this is the case, and setting this study in the wider context, if widespread cell stress and mitochondrial dysfunction are indeed clinically meaningful this may provide a direct challenge to the amyloid hypothesis (23) amidst the amyloid centric field of AD and disease modification.

Going deeper in the detail of the synaptic microstructure, I was able to characterise cortical microstructure and synaptic loss in AD in chapter 5. I found focal decreases in brain volumes were associated with synapse loss, most prominently in the hippocampus. I showed measures of extracellular free water showed large relative increases in AD compared to controls, with small increases in neurite density and focal decreases in ODI. As a result of this study, I believe that NODDI MRI could be more sensitive to differences in a pathology related to synapse loss and more widely available and could be used to support early-stage evaluations of novel

therapeutics for AD and alongside cortical volume change for stratification and monitoring of disease progression in people with AD.

Studying this particular population of patients with AD was very challenging, and was made harder by a world pandemic. I had a short window of time to bring them back for longitudinal follow up safely. The imaging study procedures were demanding and required considerable effort from the AD patients and families/carers. Given the nature of underlying illness and the episodic memory deficits in particular, I had to think more about and conduct capacity to consent to both enrolment and ongoing participation in a sensitive way. Some participants could not tolerate multiple scans, particularly at follow up, and one participant's AD had deteriorated that they could not return to the study. I had to consider various ethical issues (189) such as both the risks and benefits to an individual for taking part and build into the studies what I could and could not disclose. Together the remaining population may have limited my ability to fully characterize the disease.

Future Directions

Effective AD patient care and prevention requires the correct treatment or intervention, delivered at the optimum time point in the right patient. In AD not only is it important to classify and stage the disease, but also have a biomarker that is more sensitive to change in a clinically meaningful pathway that has the potential to be changed to avert the suffering associated with it.

A multi-modal and highly phenotyped study aimed at investigating AD in its earliest stages (ie the initial trigger points of pathology in an earlier healthy population) that are genetically and environmentally stratified (e.g. by polygenic risk score) at varying risk of AD may prove useful. This would allow the investigation and development of more accurate diagnostic and prognostic factors, and provide a platform for novel therapies to be trialled with longer longitudinal follow up to assess the impact on the neuropsychiatric signs and symptoms of the disease.

Technical advances in imaging modalities will allow in the future some of these goals to be fulfilled. (190) The promise of higher resolution in next generation PET scanners may give the ability to measure small nuclei. 10-20 fold greater sensitivity will allow rapid neurotransmitter kinetic detection and target change, lower radiation dose will facilitate acceptability, and full body scanning will allow bridging of the brain-body interface. New PET tracers and harnessing multimodal data sharing from large consortia will help these aims.

Building on this thesis, incorporating imaging markers that are responsive to cell stress, mitochondrial dysregulation and synaptic loss, with inflammatory markers at all levels of the cellular phase of AD (from molecules to systems), alongside specific MR measures such as free water would help to achieve this ambition. If such a cohort was formed it could be a potential paradigm shift in recapitulating the

disease framework for classification. Specifically, this investigation should prioritise identification of *in vivo* vulnerable homeostatic mechanisms across the time frame of AD that decompensate in both early and late stages leading to the deleterious neuropsychiatric symptoms of this disease.

Chapter 7

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